

# Biothermodynamics, Chances and Problems

J.U.Keller, Inst. Fluid-and Thermodynamics  
University of Siegen, 57068 Siegen, Germany  
keller@ift.maschinenbau.uni-siegen.de

## 1. Biothermodynamics

Overview, Historical Remarks

## 2. Structure of Thermodynamics

## 3. Biomolecules and Biofluids

DMPC-EOS (E2)

## 4. Proteins

Denaturation (E3), Adsorption (E4),  
Aggregation

## 5. Metabolism of Bacteria

Allometry, Thermal Limits of Life

## 6. Biocalorimetry

Medical Application (E6)

## 7. Bioreactors

Fermentation of Wine (E6)

Sterilization Process (E7)

## 8. Downstream – Processing

Literature

# **Biothermodynamics (BTH):**

**Application of Thermodynamics, i.e. Thermostatistics (TST) and Thermodynamics of Irreversible Processes (TIP) to biological and bioengineering Systems.**

**Biotechnology (BT): Technology using living systems like cells, bacteria, fungi etc. as chemical reactors.**

White BT	Industrial sized biocatalytic processes (fermentation) Breweries, Production vitamine B12, steroid hormones etc.;
Green BT	Plants and transgene variations for production of biofuels etc. in biorefineries;
Red BT	Medical applications of substances and processes related to living organisms, as for example interferones etc. (cancer, viruses)
Yellow BT	Pharmaceutical molecules, recombinant proteins, penicilline and other fungi;
Blue BT	Seawater based microorganisms as reactors; extremophiles... Extraction noble metals from seawater, production of new molecules

# Fields of Research in Biothermodynamics

**2nd Int. Symposium on Biothermodynamics  
DECHEMA, Frankfurt am Main, February 21-22, 2008**

## **Biomolecules**

- # Protein adsorption on surfaces**
- # Protein folding, interactions and stability**

## **Bacteria**

- # Active mass transport in biological membranes**
- # Thermodynamics of metabolic pathways**
- # Intracellular Thermodynamics**

## **Bioreactors**

- # Biocalorimetry**
- # Thermodynamics of downstream processing**
- # Thermodynamics in biological energy conversion processes**
- # Thermodynamic aspects of Systems Biology**

## **2.Basic Concepts of Thermodynamics\***

### **Thermodynamic System (W. Schottky, 1929)**

Boundaries, Set of Operations

### **Level of Description (Beschreibungsebene)**

Set of state variables (external, internal),  
Set of exchange processes and dynamic equations,  
Set of equations of state

### **1st Law of Thermodynamics and concept of Energy**

Conservation of energy and mass (E.Noether, ca. 1930)

### **2nd Law of Thermodynamics and concept of Entropy**

Law of large numbers, Central limit theorem (van Kampen, J.Meixner, 1960- )

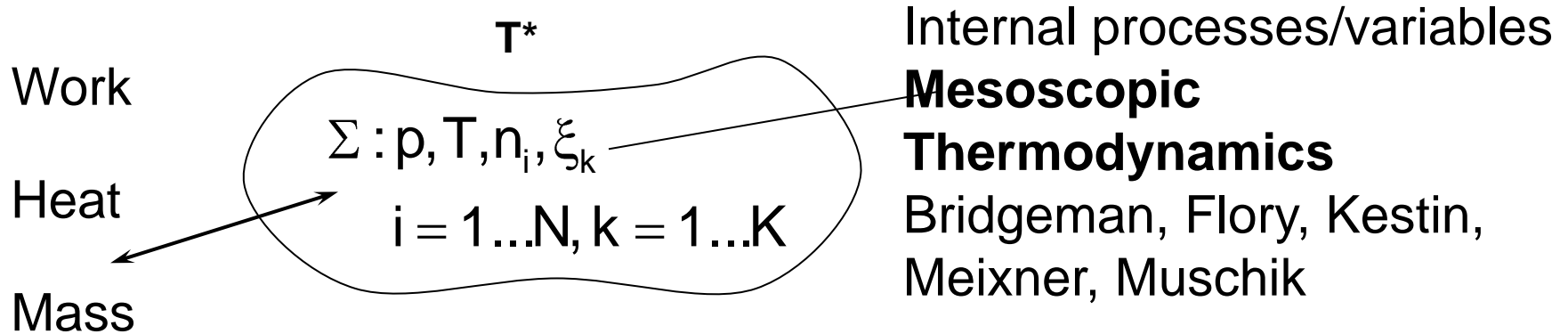
### **3rd Law of Thermodynamics**

( W. Nernst, M. Planck, ca. 1910)

\*Thermodynamics: Phenomenological theory of many – particle - systems.

# Thermodynamic System (W. Schottky, 1929)

$\Sigma$ : Set of bodies surrounded by well defined boundaries exchanging with its environment ( $\Sigma^*$ ) by external operations transfer energies as



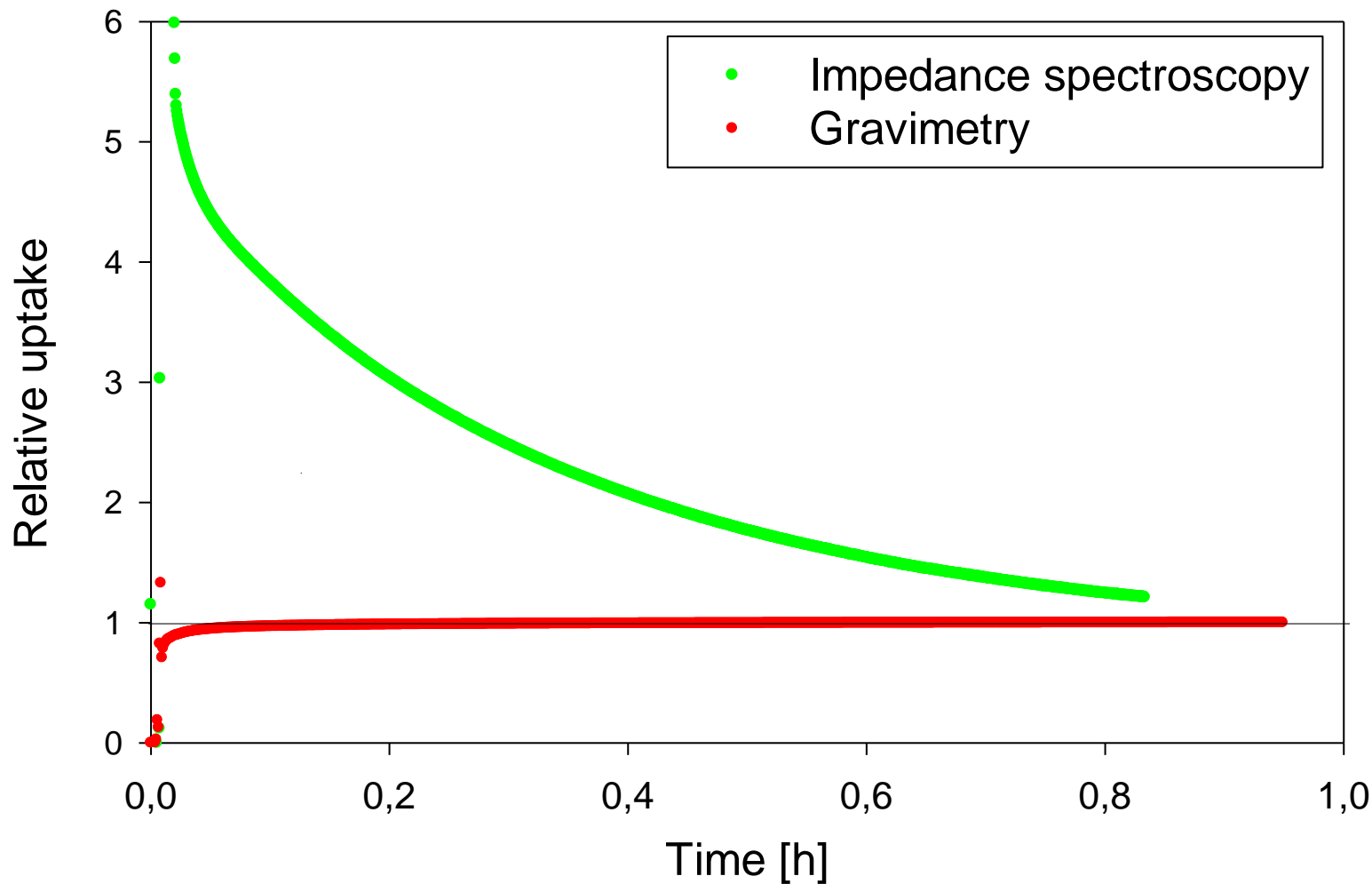
.....  $\Sigma^* : p^*, T^*, h^{(\alpha)}, s^{(\alpha)}, \mu_i^{(\alpha)}$

Information  
(Living Systems)  $\alpha = 1 \dots A$

External & Internal Processes: Level of macroscopic description or state of system ( $\Sigma$ ).

# **Internal Variables of Thermodynamic Systems, Examples**

1. Glass: Transition Processes: amorphous phase → crystalline phase
2. Polymeric materials: Molecular relaxation processes
3. Gases & Liquids: Slow dissociation / recombination processes  
(radioactive decay) ( $\text{H}_2\text{S}/\text{AC}$ )
4. Liquid crystals: Phase transition processes
5. Dielectric-/Diamagnetic relaxation processes
6. Proteins in (ionic) solution: Structural- / Molecular-relaxation  
(denaturation- i.e. folding, unfolding processes)



**Uptake curves of H<sub>2</sub>S on MS 13X, T=298K**

## Gibbs Equation for $G = G(T, p, n_1 \dots n_N, \xi_1 \dots \xi_k)$

$$dG = -SdT + Vdp + \sum_{i=1}^N \mu_i dn_i - \sum_{k=1}^K A_k d\xi_k$$

Chemical reactions ( $Q \leq N - E$ )

$$C_i = \sum_{e=1}^E \alpha_{ie} E_e, \quad \sum_{i=1}^N \nu_{iq} C_i = 0, \quad q = 1 \dots Q$$

Conservation of atomic numbers:

$$\sum_{i=1}^N \nu_{iq} \alpha_{ie} = 0, \quad e = 1 \dots E, \quad q = 1 \dots Q$$

Chemical production of component (i):

$$n_i^c = n_i^* + \sum_{q=1}^Q \nu_{iq} (\gamma_q - \gamma_q^*), \quad i = 1 \dots N$$



## Gibbs Equation (T = const, p = const)

$$dG = - \sum_{q=1}^Q A_q^c d\gamma_q - \sum_{k=1}^K A_k d\xi_k$$

$$A_q^c = - \sum_{i=1}^N \mu_i \nu_{iq}$$

a) Restricted or frozen equilibria:  $\xi_1 \dots \xi_k = \text{const} \dots$  arbitrary value

$$A_q^c(T, p, \gamma_1 \dots \gamma_Q, \xi_1 \dots \xi_k) = 0, \quad q = 1 \dots Q$$

Reaction numbers  $\rightarrow \gamma_{qE} = \gamma_q(T, p, \xi_1 \dots \xi_k)$

b) Full or unrestricted equilibria:

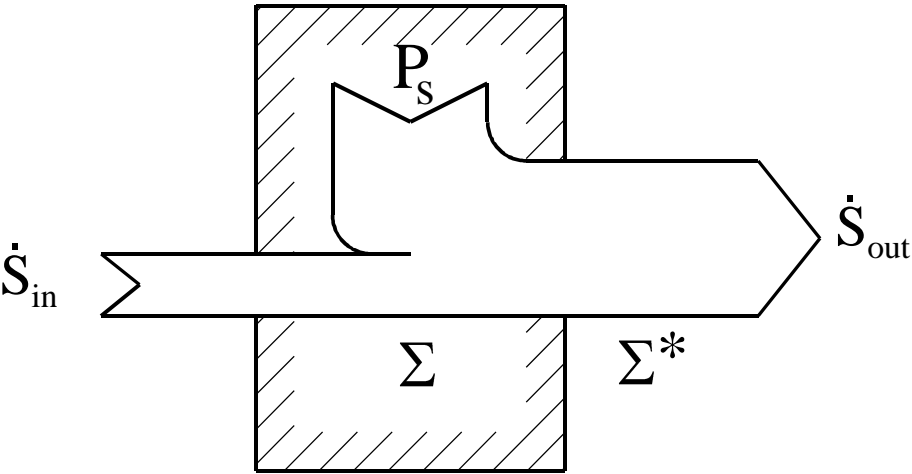
$$A_q^c(T, p, \gamma_1 \dots \gamma_Q, \xi_1 \dots \xi_K) = 0, \quad q = 1 \dots Q$$

$$A_k(T, p, \gamma_1 \dots \gamma_Q, \xi_1 \dots \xi_K) = 0, \quad k = 1 \dots K$$

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$$\rightarrow \gamma_{qE} = \gamma_q(T, p), \quad \xi_{kE} \dots \xi_k(T, p)$$

# Thermodynamics of Photosynthesis (E1)



Evaporation of Additional Water:

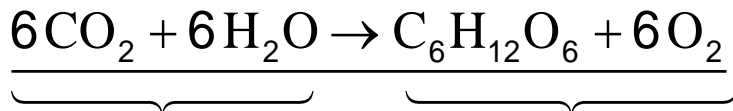
$$\dot{S}_{in} = \dot{S}_{out} + 0.24 \frac{\text{kJ}}{\text{mol K}} \cdot \dot{n}_{GL}$$

$$2.2 | \dot{S}_{H_2O_L} = \dot{S}_{H_2O_V} - 0.11 \frac{\text{kJ}}{\text{mol K}} \cdot \dot{n}_W$$

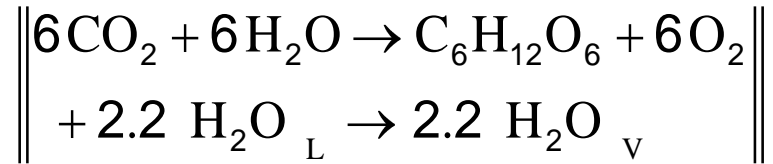
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$$\dot{n}_W = 2.2 \cdot \dot{n}_{GL}$$

E. Schrödinger (~1940)



$$\dot{S}_{in} = \dot{S}_{out} + 0.24 \frac{\text{kJ}}{\text{mol K}} \cdot \dot{n}_{GL}$$



2<sup>nd</sup> Law:  $\dot{S}_{in} \leq \dot{S}_{out} \quad ?$

### 3. Biomolecules and Biofluids

**Biomolecules** (proteins,enzymes etc.,aggregates of amino acids (MBM)  
Spatial structure ...Stereochemistry,  
Surface: polar & non-polar regions, electric charges.

**Solvent molecules** (water,alcohols,organc solvents etc. ) ( Mw <<< MBM )  
polar & non-polar fluids, salts (ions)

**Solvent molecule near surface of biomolecule is different from solvent molecule in the bulk phase.**

Problems: Biomolecules as „subsystems“ of biofluids ?

Surface of biomolecule as sorbent for solvent particles ?

„State of biomolecules“ (native,denatured, etc.) ?

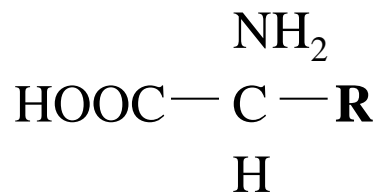
Interactions between biomolecules

Thermodynamics: **Internal Variables** of a system....**internal equations of state**

**Amino Acids (AA) Selection (1), Bohinski (1979), Voet&Voet (1996)**

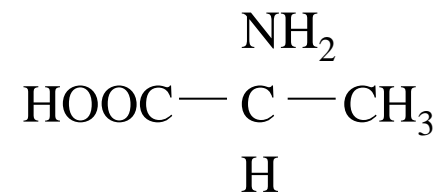
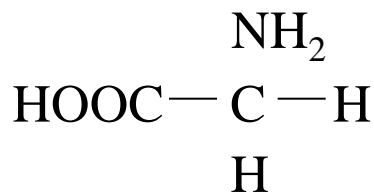
General Structure

Aliphatic AA



Glycine (gly)

Alanine (ala)



R-Group:

Aliphatic

Aromatic

Hydroxyl

Acidic

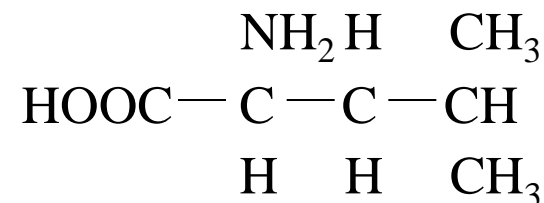
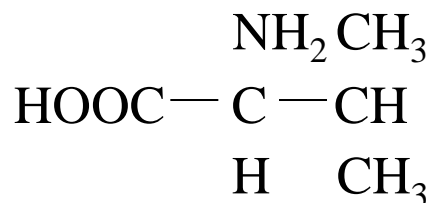
Basic

Imino

Sulfur

Valine (val)

Leucine (leu)



etc.

A – Chain

Gly – Ile – Val – Glu – Gln – Cys – Cys – Thr – Ser – Ile – Cys – Ser – Leu – Tyr – Gln

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

B – Chain

Asn – Cys – Tyr – Asn – Glu – Leu

21 20 19 18 17 16

Phe – Val – Asn – Gln – His – Leu – Cys – Gly – Ser – His – Leu – Val – Glu – Ala – Leu

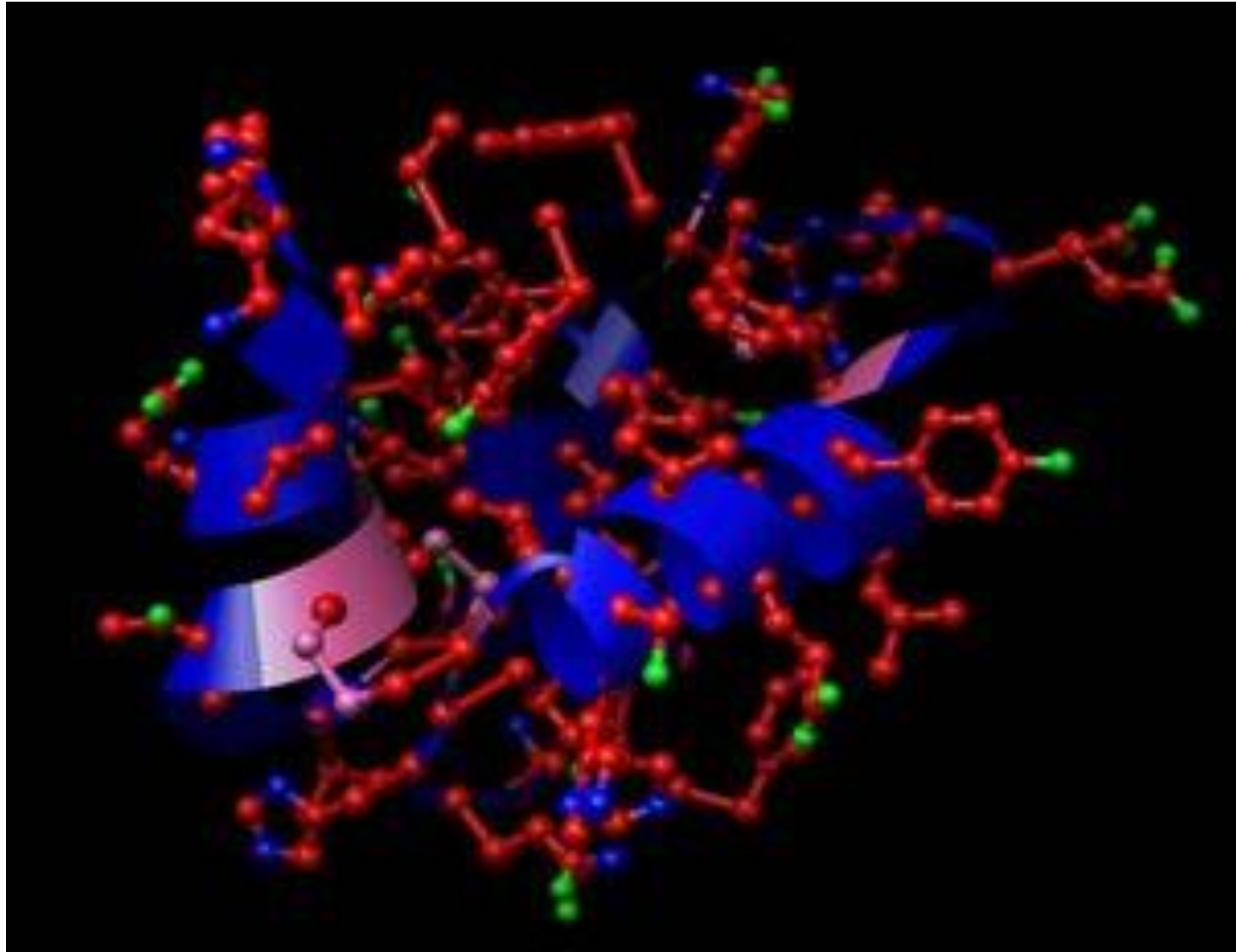
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Thr – Lys – Pro – Thr – Tyr – Phe – Phe – Gly – Arg – Glu – Gly – Cys – Val – Leu – Tyr

30 29 28 27 26 25 24 23 22 21 20 19 18 17 16

Primary Structure of Human Insulin (Roempp)

Polypeptide (A, B), M  $\cong$  6000 D



**Insulin-Molecule**, Source: Wikipedia 2005

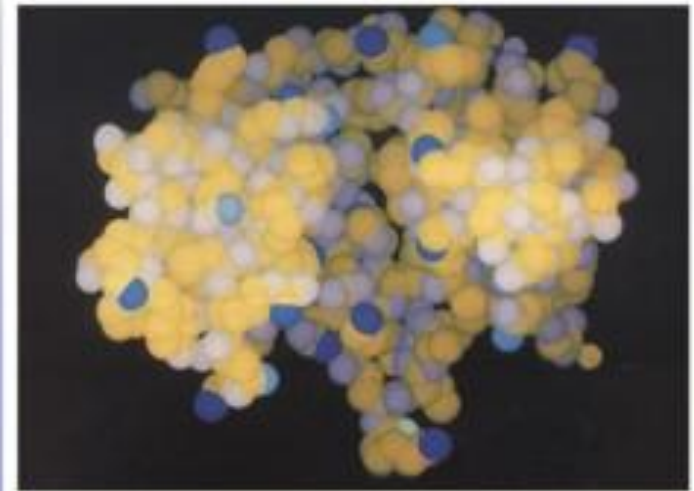
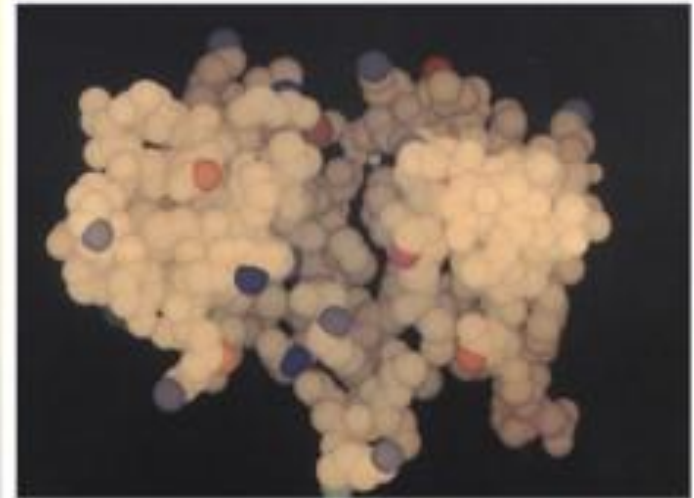
# proteins

## computer graphics of lysozyme

Re: W. Norde,  
Colloids and Interfaces in  
Life Sciences, 2005



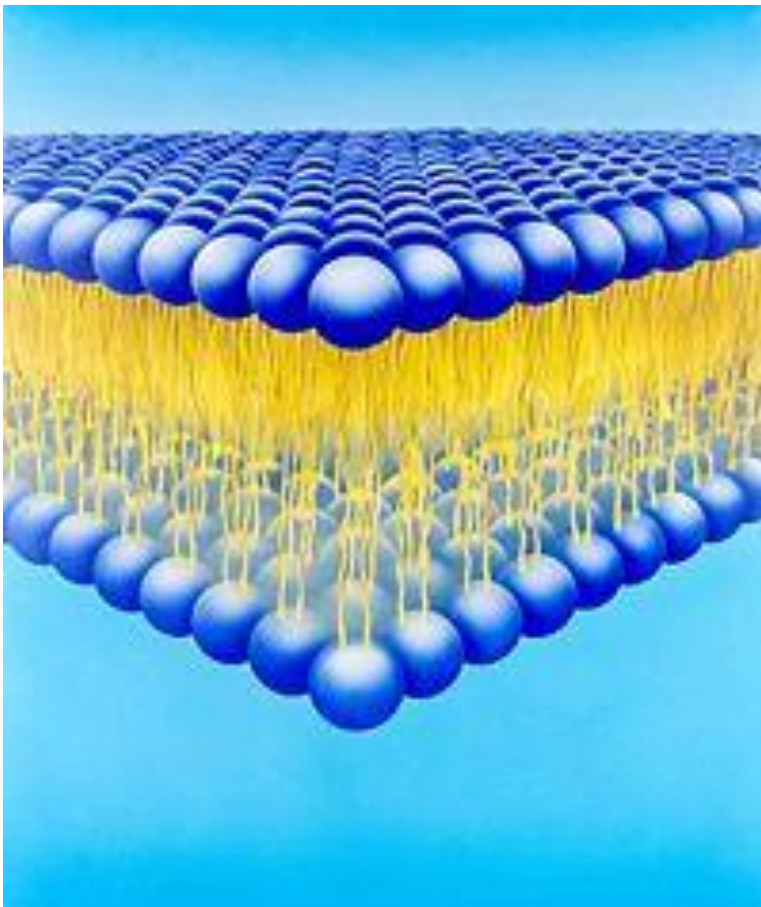
secondary structure



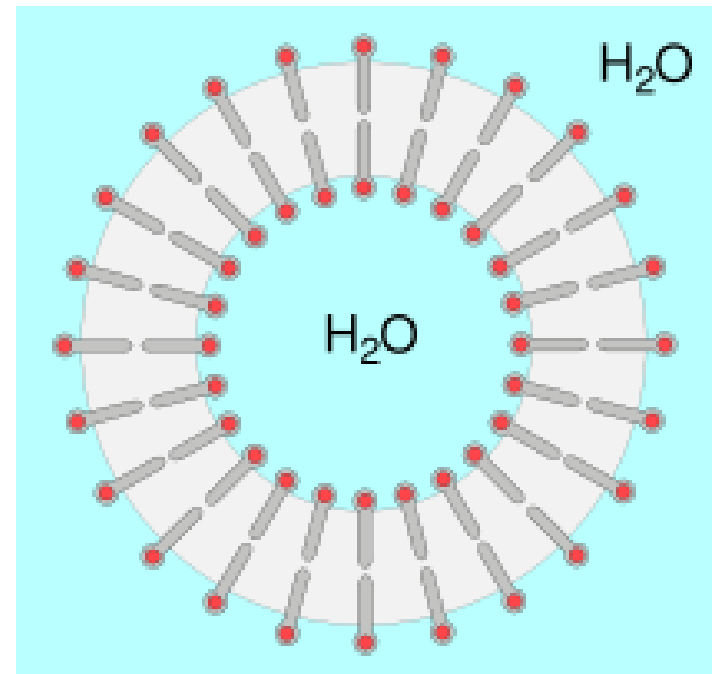
tertiary structure

# Cell Membranes: Thermal Equation of State (E2)

Double layer of lipid molecules  
Polar „heads“ – Non-polar „tails“

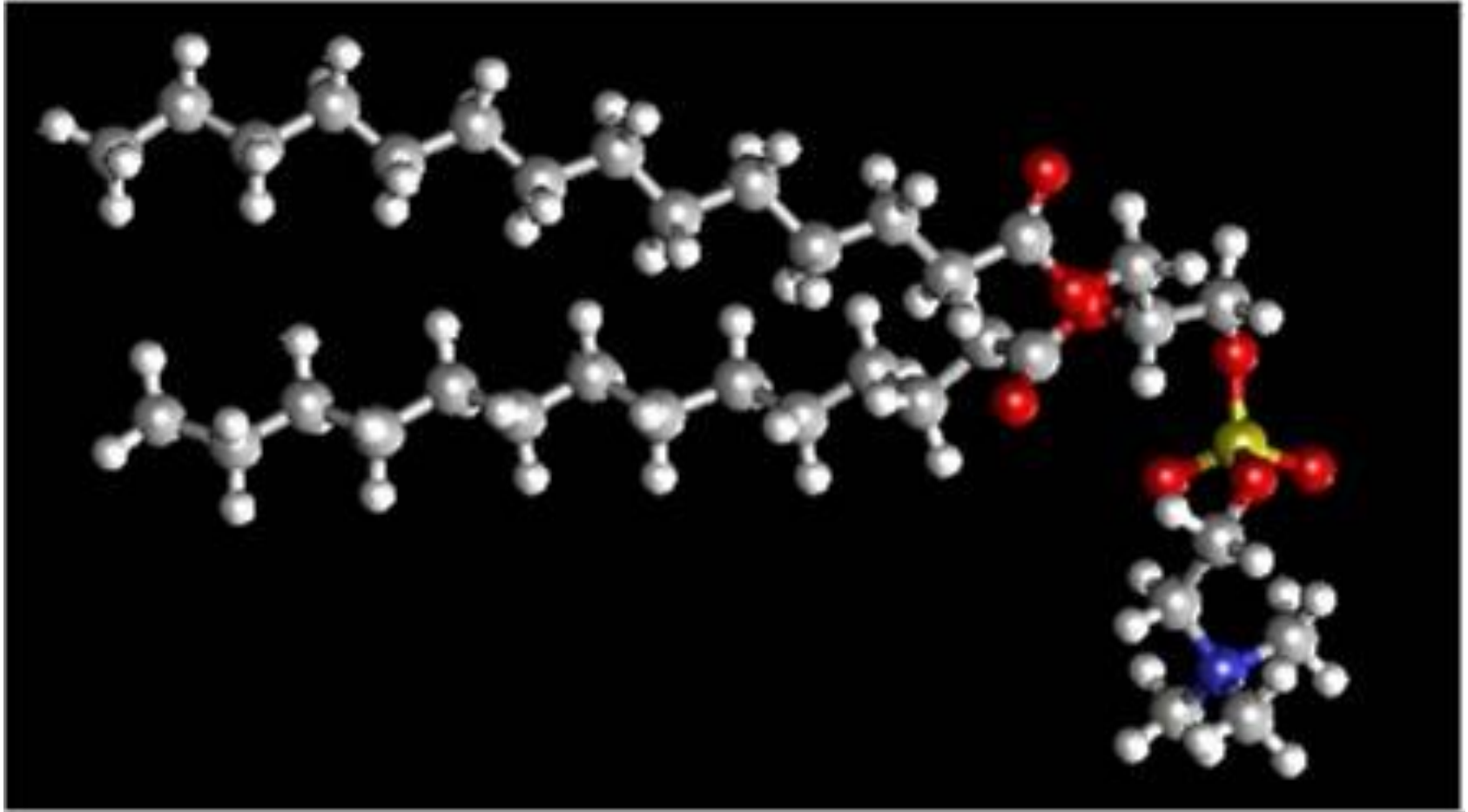


Lipid bilayer forming a micelle



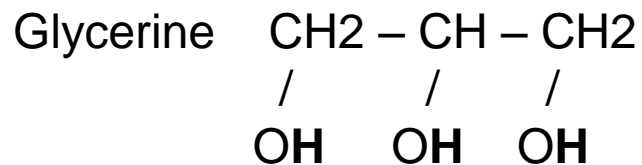
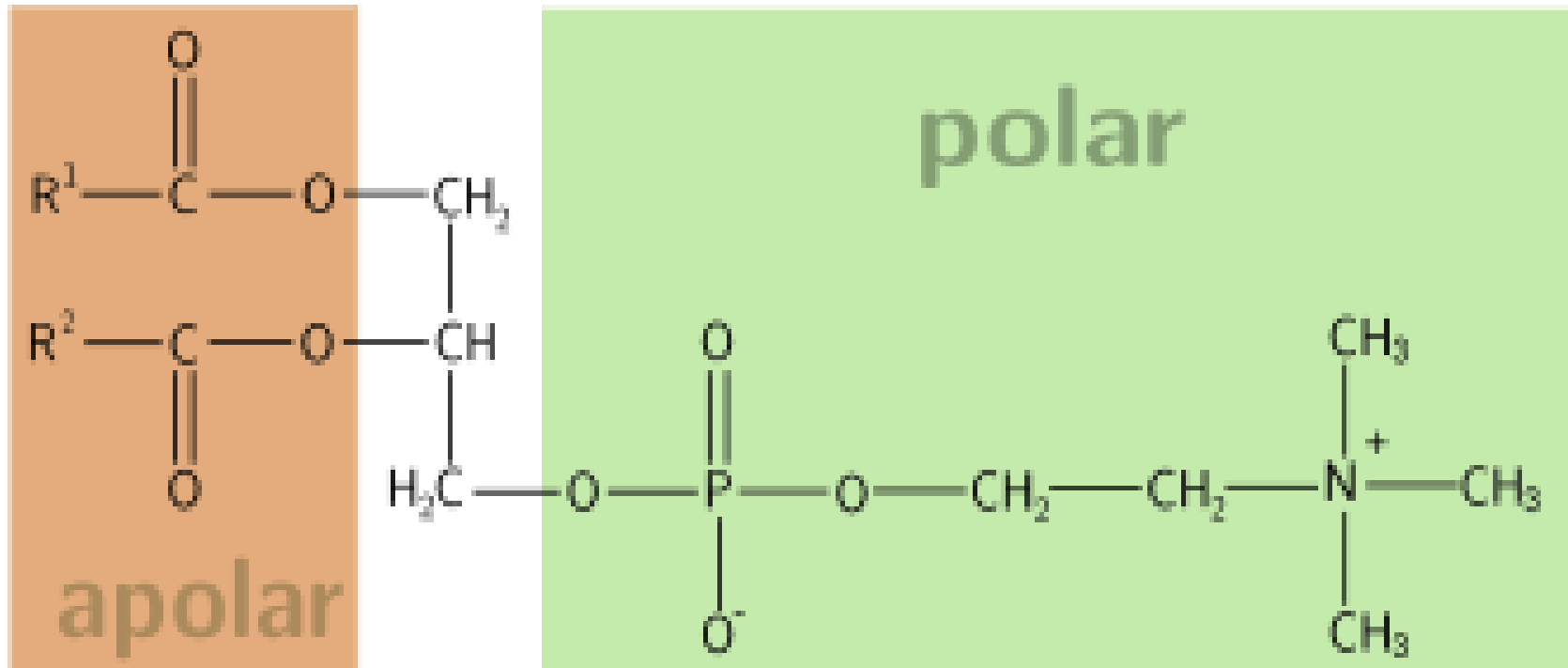


# 1,2-Dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC)



# DMPC – Structure: Phosphatidylcholine / Lecithine

Fatty acids

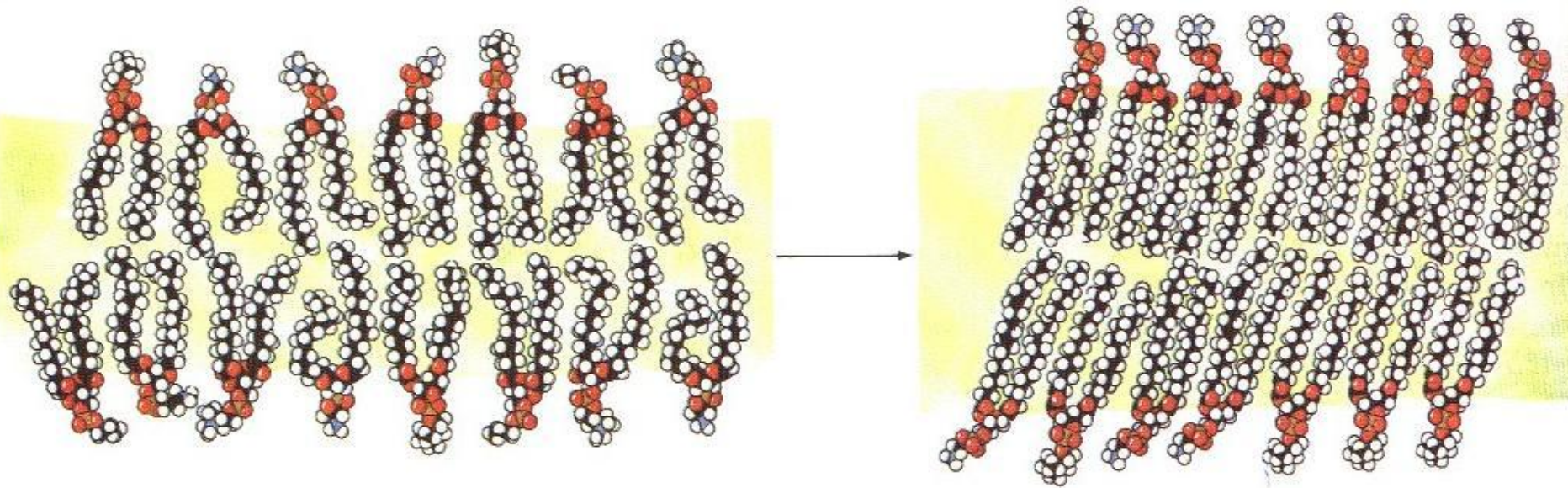


Choline

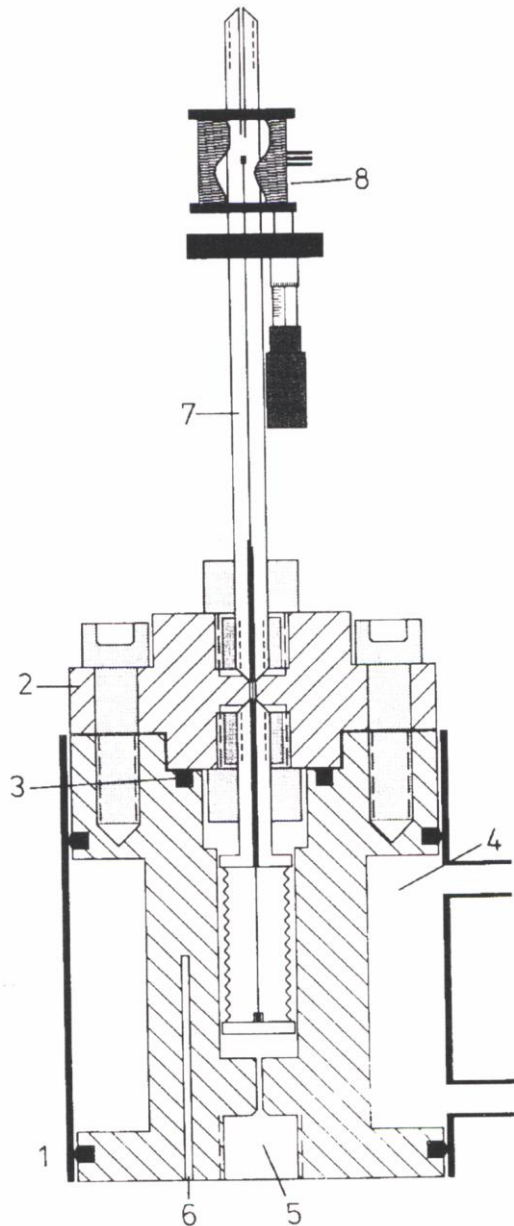
# Lipid Membranes, Phase Transition Fluid - Gel

$T > T_t(p, \dots)$

$T < T_t(p, \dots)$



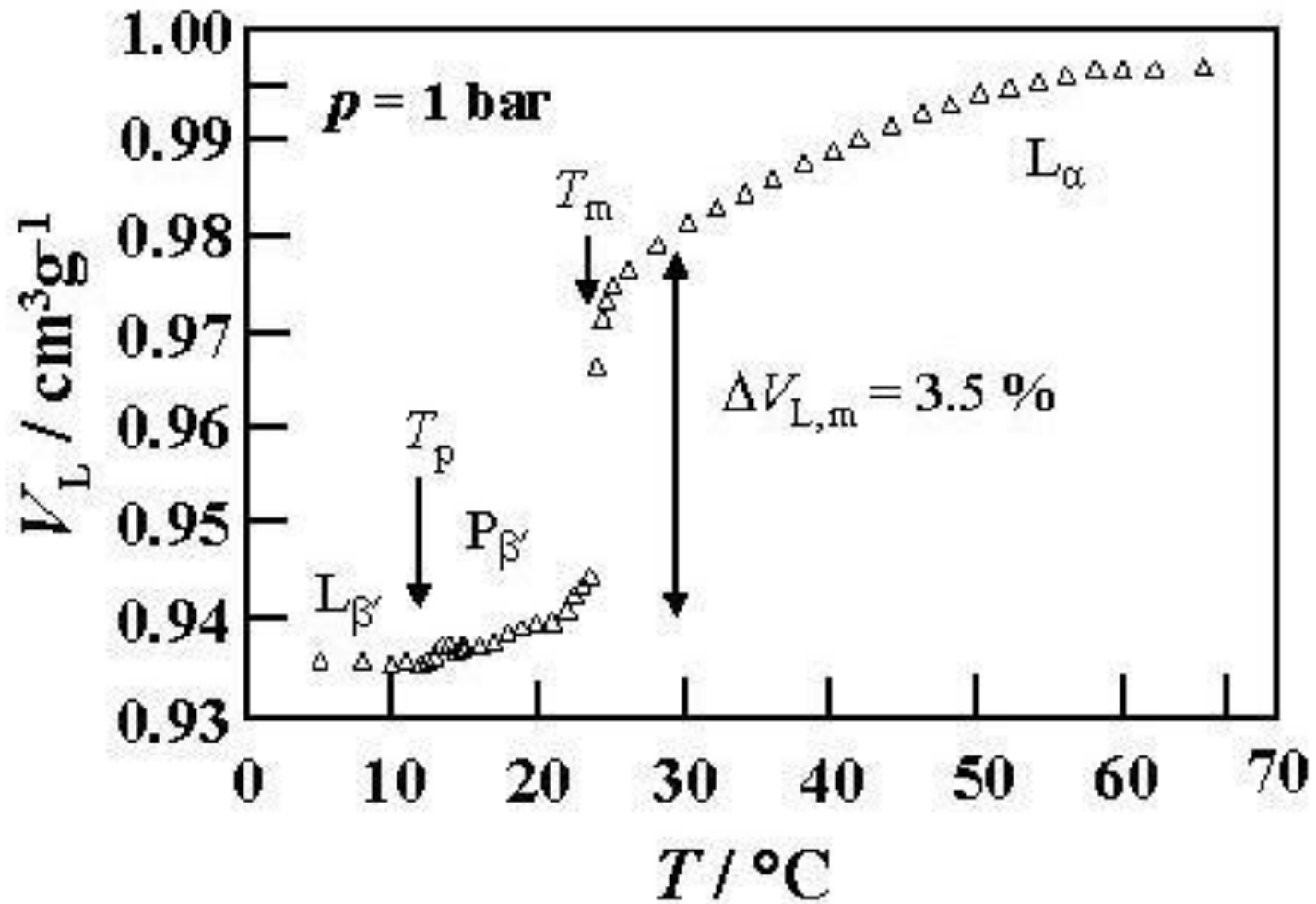
Lipid by layer formed of phosphatidylcholine (Voet&Voet, p. 288)



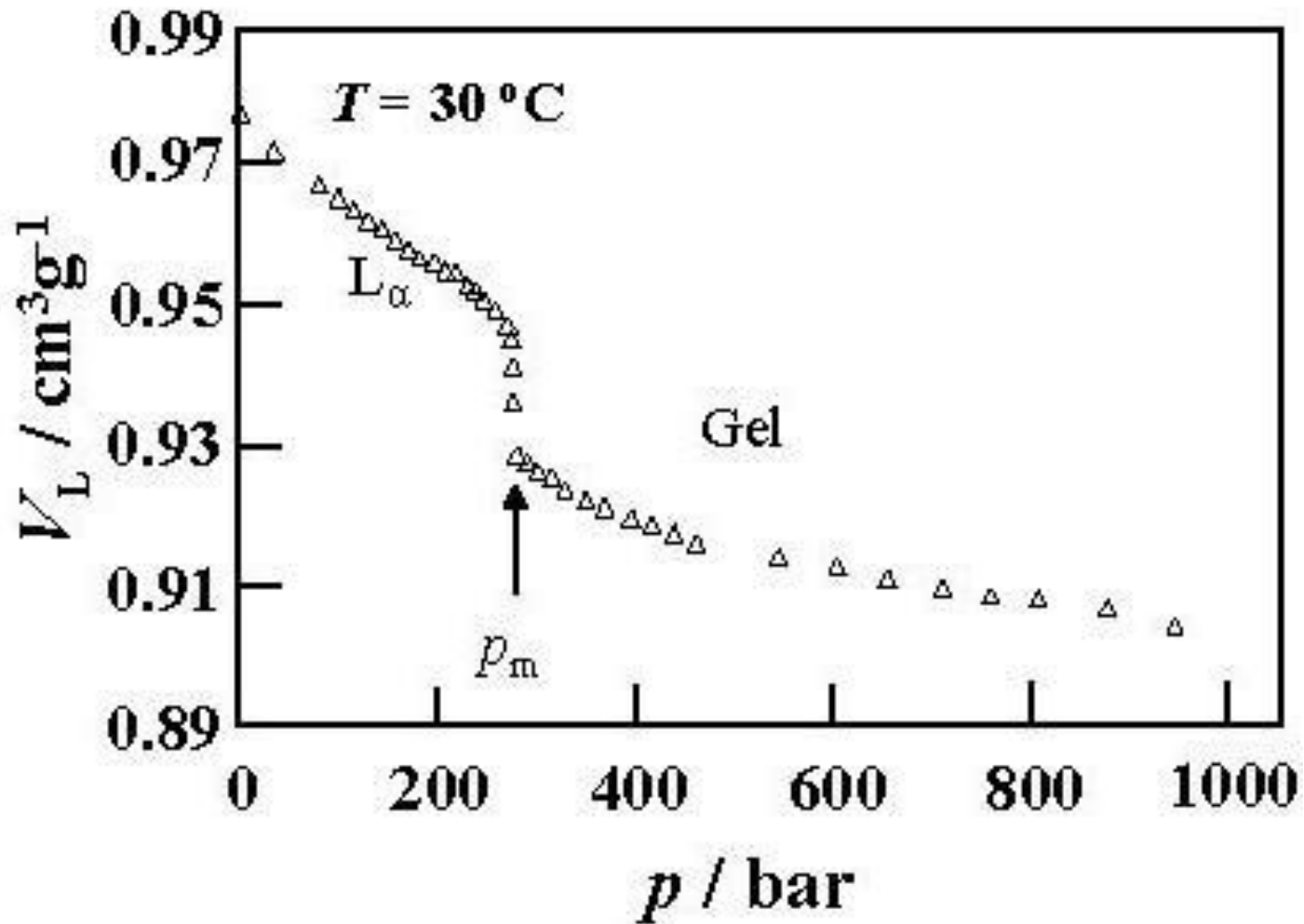
## Volumetric Cell $T = (0 - 100) \text{ C}$ $P < 250 \text{ MPa}$

- 1 Pressure cell
- 2 Top flange
- 3 Viton O-ring
- 4 Thermostate
- 5 High pressure nut
- 6 Thermocouple inlet
- 7 High pressure pipe
- 8 Inductive coil

Ref. Böttner M. et al., High Pressure Volumetric Measurements on Phospholipid Bilayers, Z. Physik.Chemie 184(1994),p.205



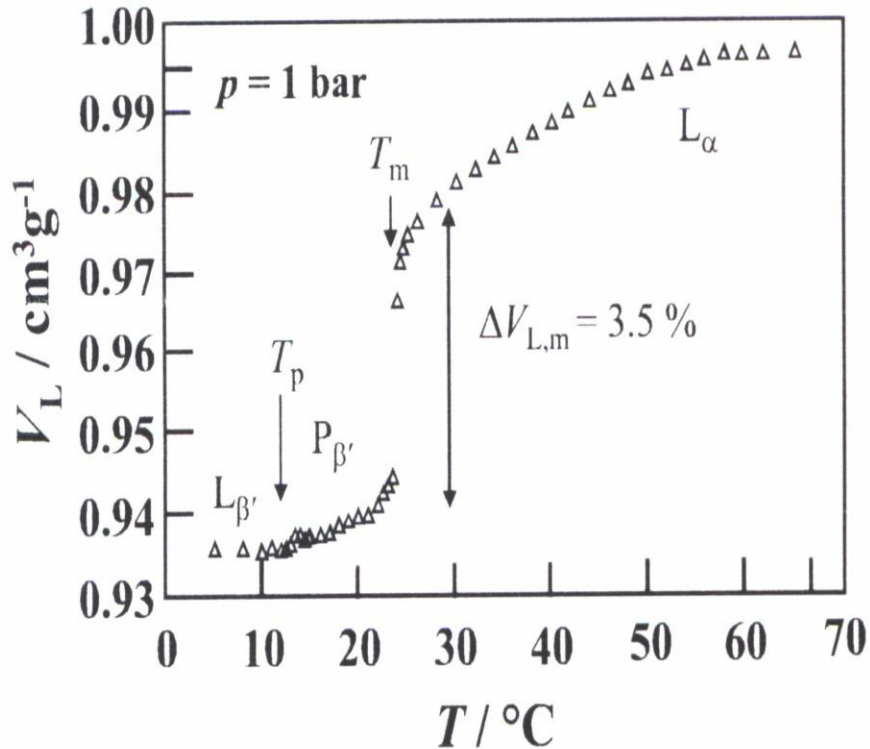
Temperature and pressure dependence of the specific volume of DMPC\*) in water. (R. Winter, JNE 6-22, 2007) \*)1,2-dimyristoyl-s,n-glycero-3-phosphatidylcholine



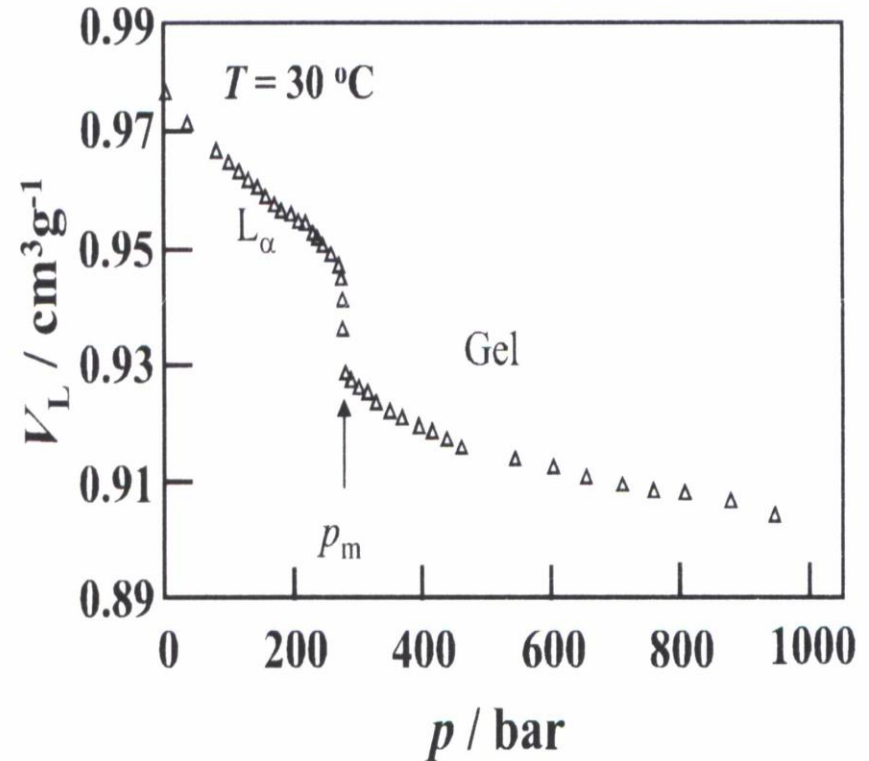
Temperature and pressure dependence of the specific volume of DMPC\*) in water. (R. Winter, JNE 6-22, 2007) \*)1,2-dimyristoyl-s,n-glycero-3-phosphatidylcholine

# p,v,T- Data of DMPC Bilayers, Phase Transition Fluid-Gel

T,v-Data at p=1bar



p,v-Data at T=30 C



Measurement Method: High Pressure cell, volumetry.

Ref.: R.Winter et al., JNE 32(1), 2007, p.41

# DMPC Thermal Equation of State (EOS)

Aliphatic tails of DMPC-molecules may aggregate/adsorb on each other.

Degree of aggregation:

Free volume

$$\alpha(v) := \frac{v_0 - v}{v_0 - b_0} \quad 0 < \alpha(v) < 1$$

$$\beta(v) := \frac{v - b_0}{v_0 - b_0}$$

Fluid state      Gel state

Fractality

EOS:  $p(\alpha, T) := A(T) \cdot \alpha + B(T) \cdot \alpha^2 + D(T) \cdot \alpha^3 + C(T) \cdot \frac{\alpha^\gamma}{1 - \alpha^\gamma} \quad \gamma := 1$

Virial expansion ...

Adsorption term

$$A(T) := A_0 \cdot [1 + a \cdot (T - T_0)]$$

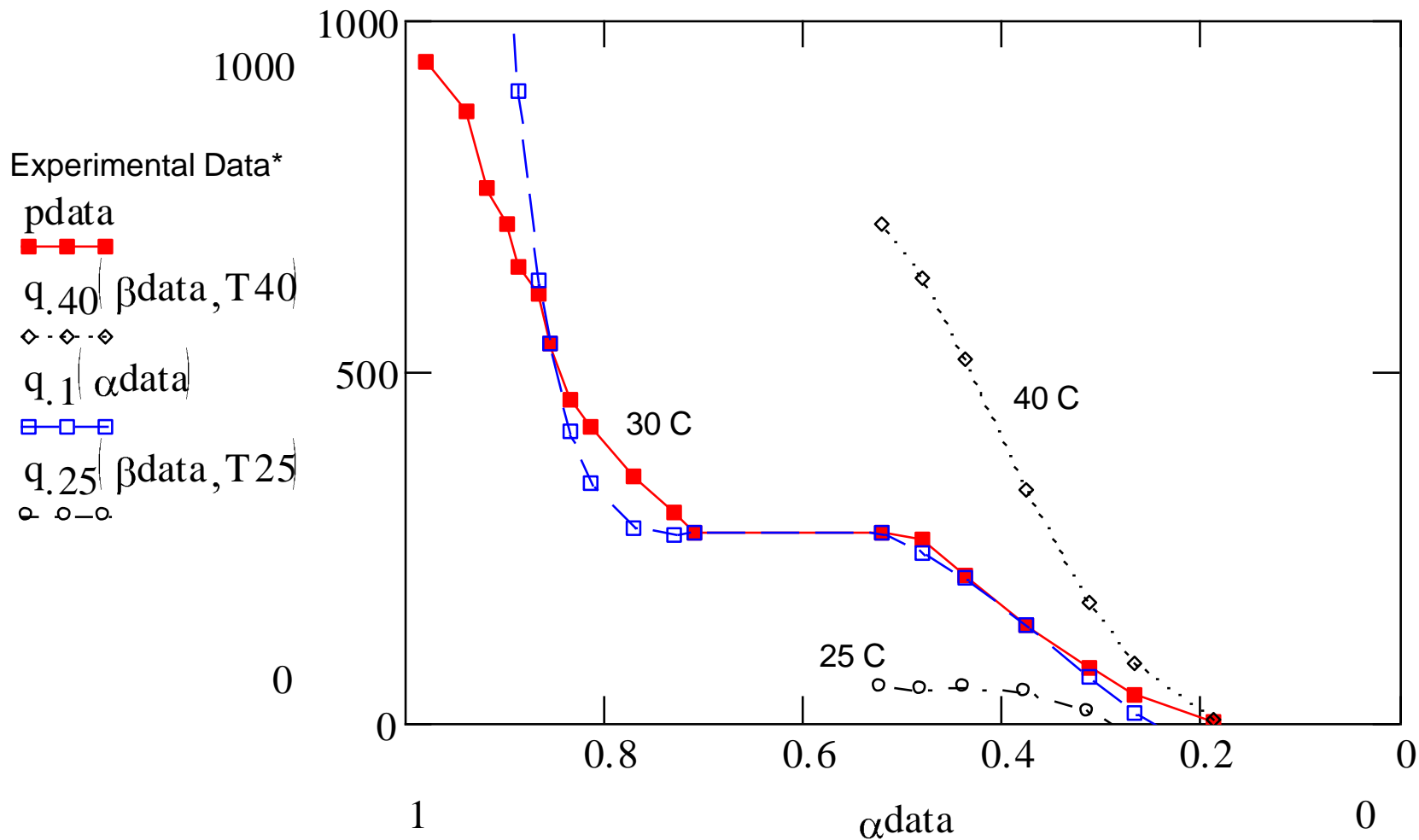
A = -1873 bar	a = -0.54
B = 7942	b = -0.051
D = -8997	d = -0.429
C = 333.34	c = -2.534

.....

$$D(T) := D_0 \cdot [1 + d \cdot (T - T_0)]$$

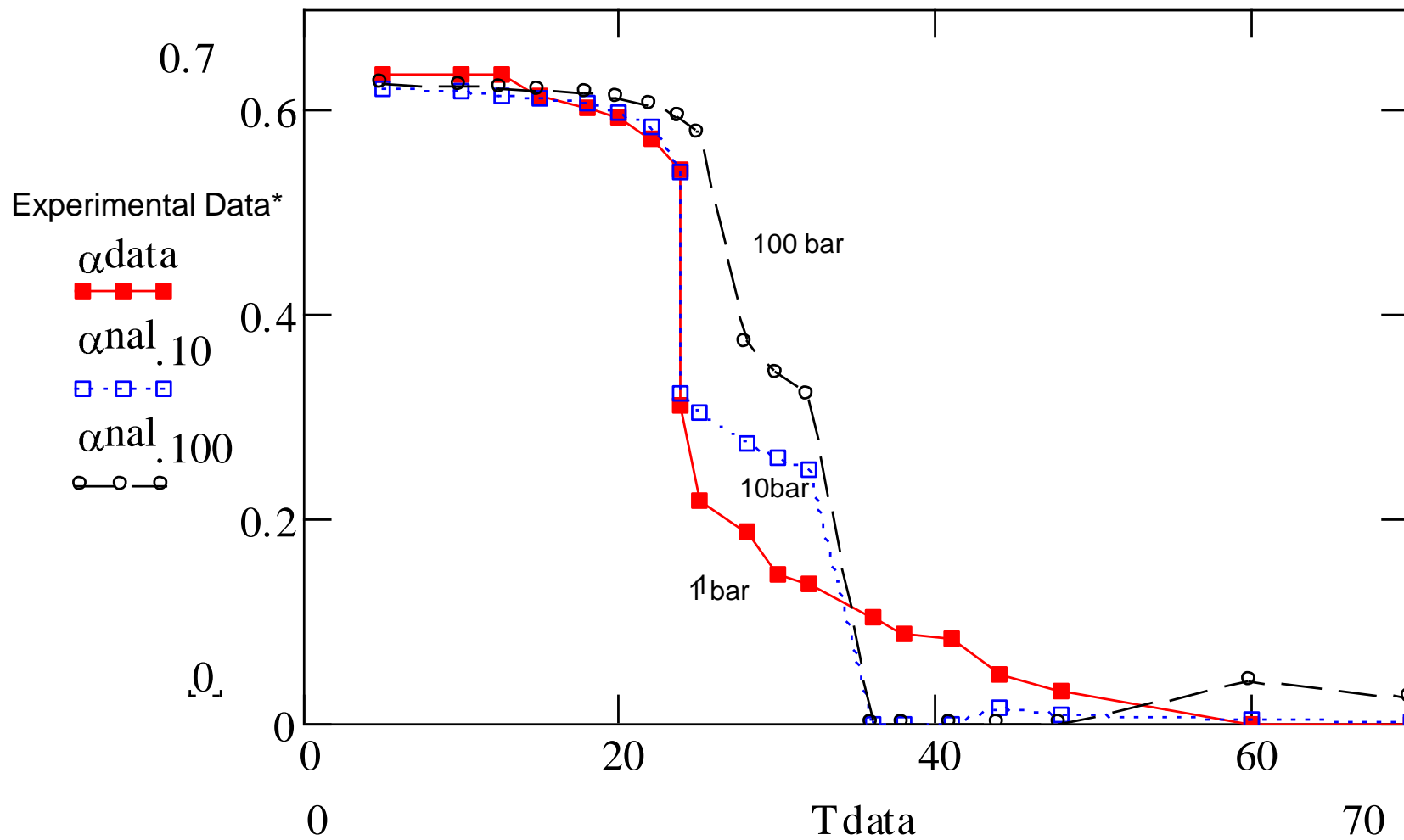


# DMPC Thermal Equation of State (EOS) Correlation of Isothermal Data



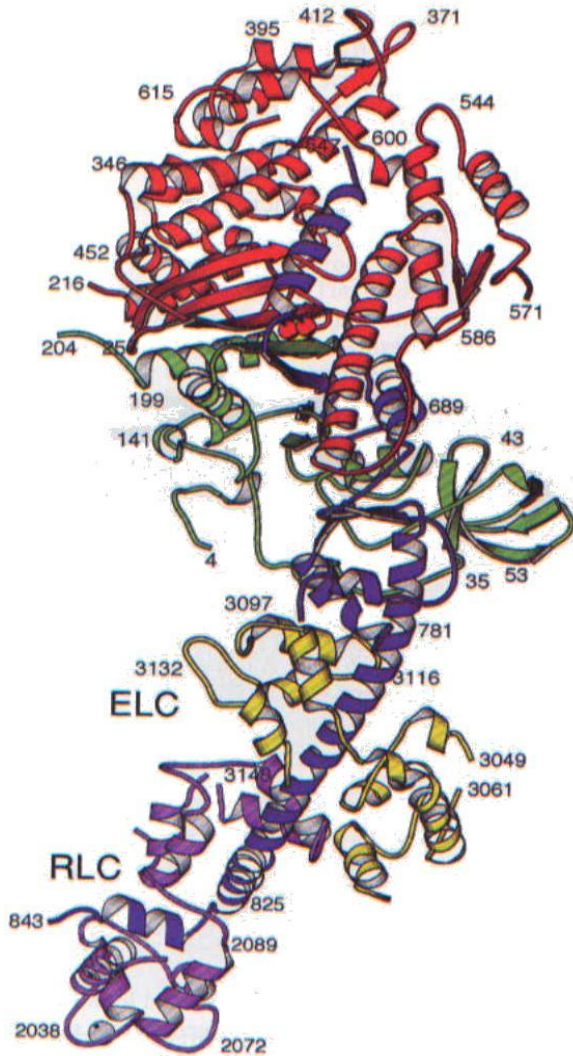
\* R. Winter et al., JNE 32(2007), p.41

# DMPC Thermal Equation of State (EOS) Correlation of Isobaric Data



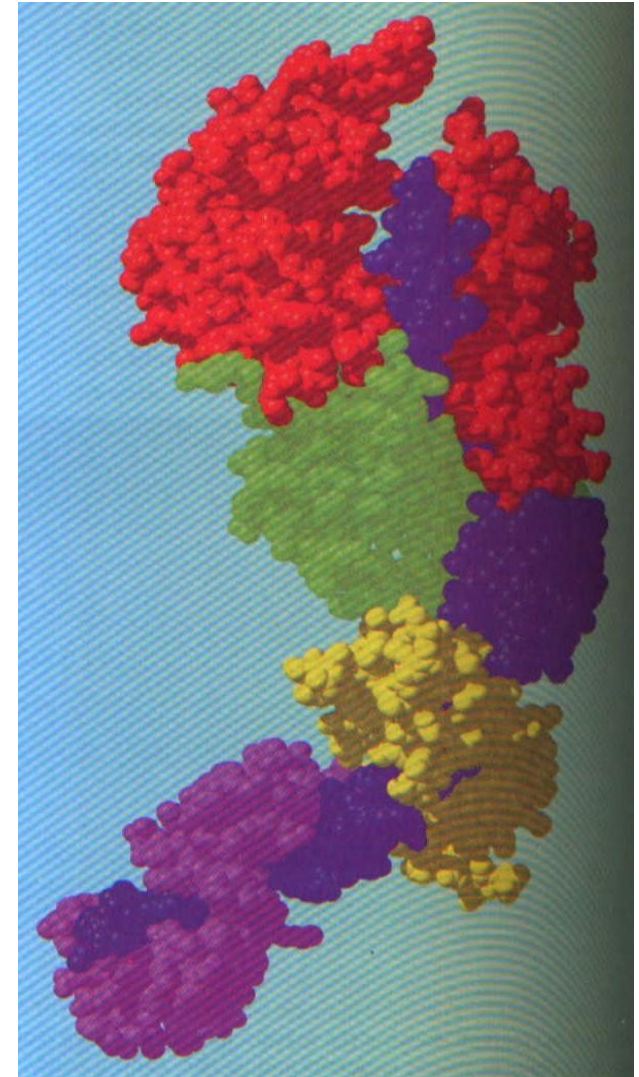
\*R.Winter et al., JNE 32(2007),p.41-

# 4. Proteins (Example): Myosin from Chicken Muscle



Secondary Structure

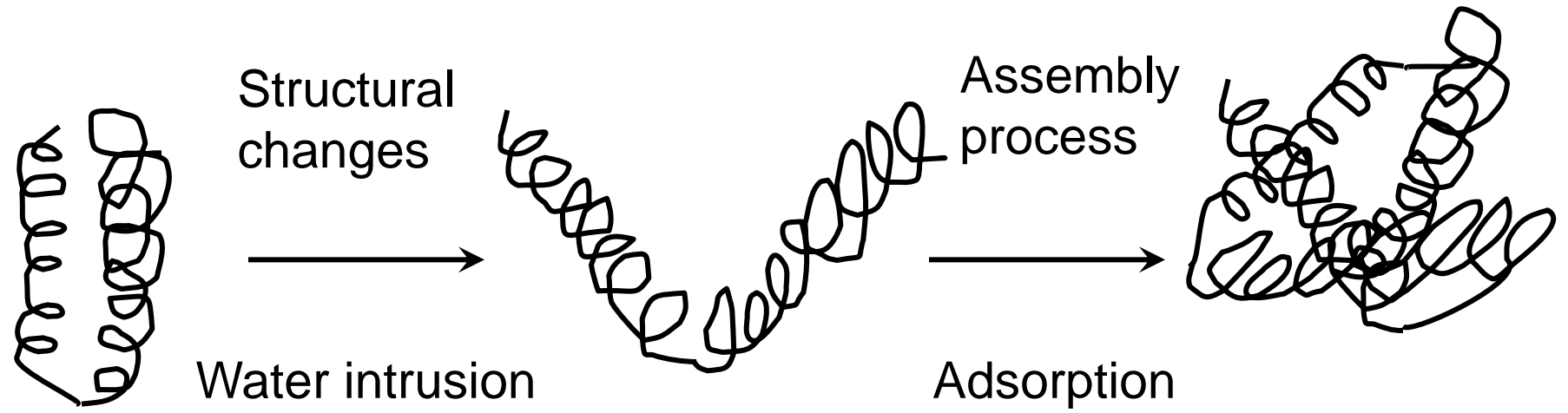
Voet&Voet  
 Biochemistry  
 Wiley,N.Y.  
 1995



Tertiary Structure (X-Ray)

# Proteins: Unfolding and Aggregation (E3) **(Alzheimer Disease)**

→ Loss of bioactivity



Native Protein (N)  
Dense packing

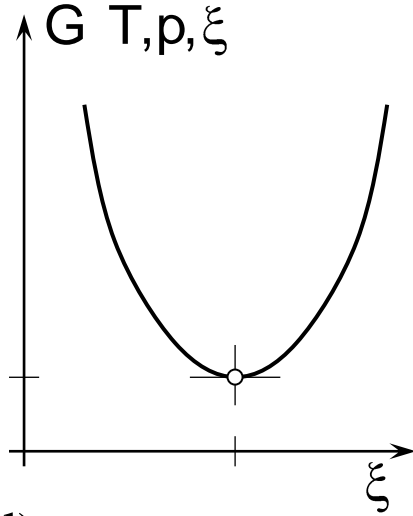
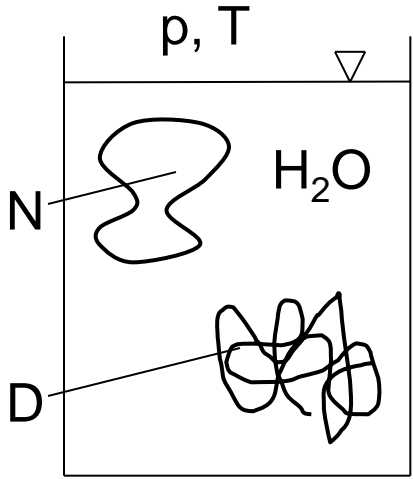
Stimulated Transition  
State, Defolding (D)

Non-native state  
Aggregation (A)  
Self-adsorption

$$M \approx 20000 D$$

$$\epsilon_r \approx 10$$

# Denaturation of Proteins, Thermodynamic Analysis, Equilibria



Reaction parameter:

$$n_N = n_{N0} - \xi, \quad dn_N = -d\xi$$

$$n_D = n_{D0} + \xi, \quad dn_D = d\xi$$

$$2,3 : dG = -\mu_N + \mu_D d\xi = 0$$

$$\rightarrow \underline{\mu_N = \mu_D} \quad 4$$

N ... Native (folded) state

D ... Denaturated (unfolded) state

$N \leftrightarrow D$  Quasichemical reaction ( $\xi$ )

$$G = G(T, p, n_N, n_D) = \mu_N n_N + \mu_D n_D \quad 1$$

$$dG = -SdT + Vdp + \mu_N dn_N + \mu_D dn_D \quad 2$$

Equilib.:  $G \rightarrow \text{Min}, T = \text{const},$

$$p = \text{const}, n = \text{const}$$

$$dG = 0, \quad d^2G > 0 \quad 3$$

$$\mu_i = \mu_{i0}(T, p) + RT \ln \gamma_i x_i, \quad i = D, N \quad 5$$

$$5, 4 \quad \underbrace{\mu_{N0} - \mu_{D0}}_{-\Delta G} = RT \ln \gamma_D x_D / \gamma_N x_N$$

$$-\Delta G = RT \ln K_{eq}(T, p)$$

$$\underline{\underline{K_{eq} \doteq \frac{\gamma_D x_D}{\gamma_N x_N} = e^{-\Delta G/RT} \quad 6}}$$

Ideal solution:  $\gamma_D = \gamma_N = 1$

Real solution: Calor. measurements

# Thermal Denaturation of Myoglobin

## Experimental Data

153 Amino acids

Seize:  $(44 \times 44 \times 25) \text{Å}^3$

Molecular Weight  $\approx 18 \text{kD}$

N ... Native (folded) State

D ... Denaturated (unfolded) State

Equilibrium at  $T = \text{const}$ ,  $p = \text{const}$

$$\Delta G_{DN} \text{ p, T} = -RT \ln \left( \frac{\gamma_D x_D}{\gamma_N x_N} \right)$$

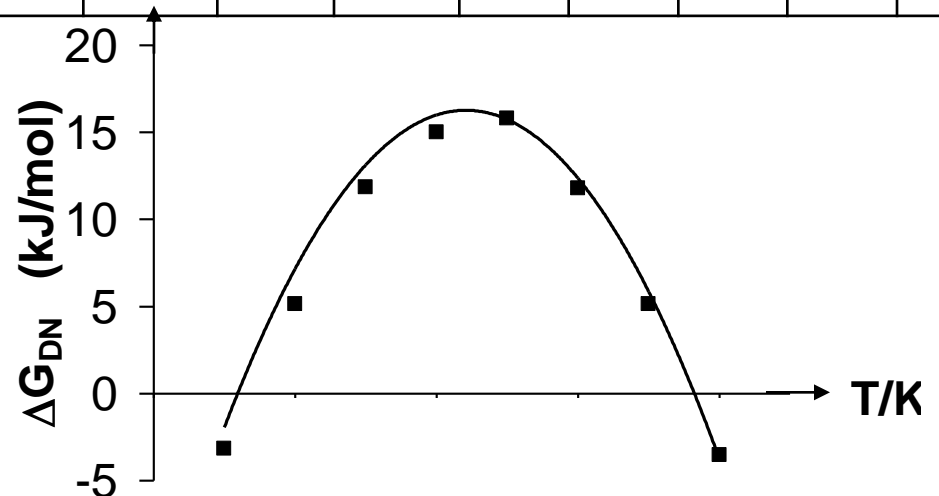
$$\Delta G_{DN} = \mu_{D0} - \mu_{N0}$$

Approx.:  $\gamma_D = \gamma_N = 1$

$$\Delta G_{DN} > 0 \rightarrow x_D \ll x_N \dots \text{N...stable}$$

$$\Delta G_{DN} < 0 \rightarrow x_D \gg x_N \dots \text{N...unstable}$$

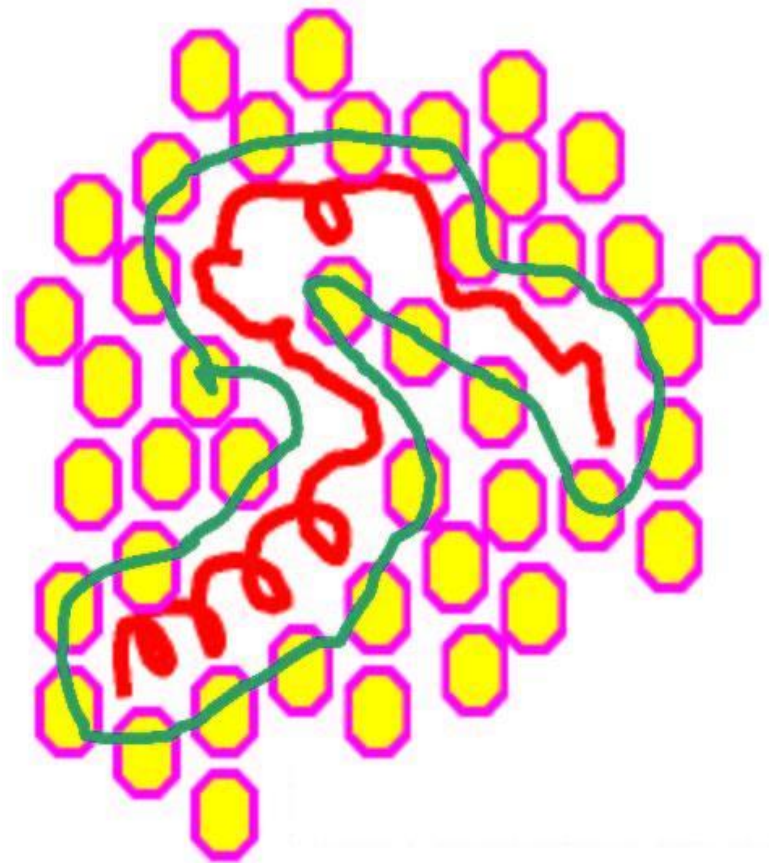
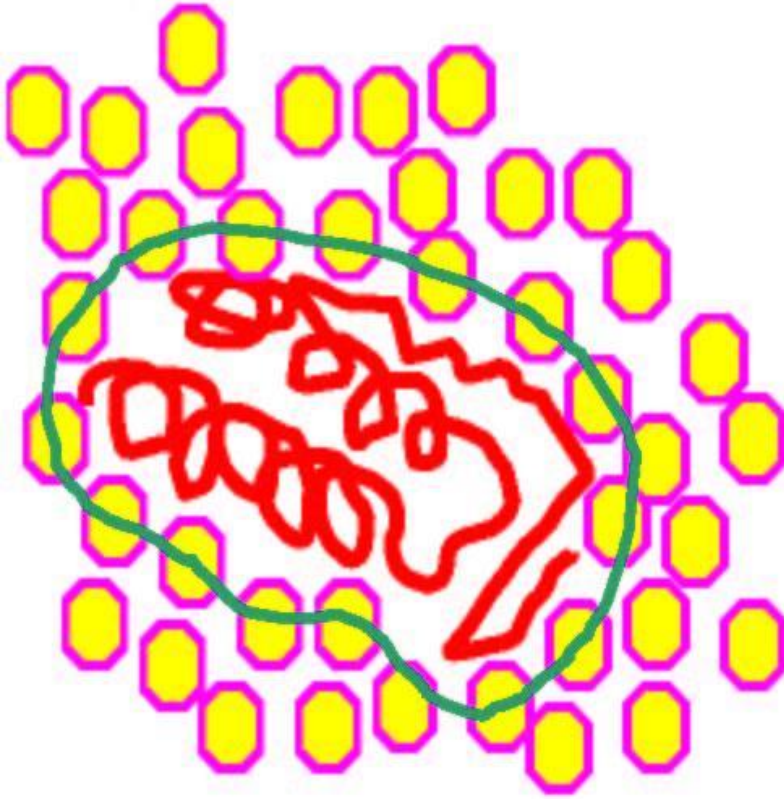
T/K	270	280	290	300	310	320	330	340
$\Delta G_{DN}$ (kJ/mol)	-3.16	5.13	11.8	15	15.8	11.8	5.13	-3.53
$\Delta H_{DN}$ (kJ/mol)								
$\Delta S_{DN}$ (kJ/mol K)								



## Protein(P) - Water(W) Interactions (E4)

P: Conformational Changes, Unfolding

W: Adsorption, Intrusion, Coating of (P): Stabilization



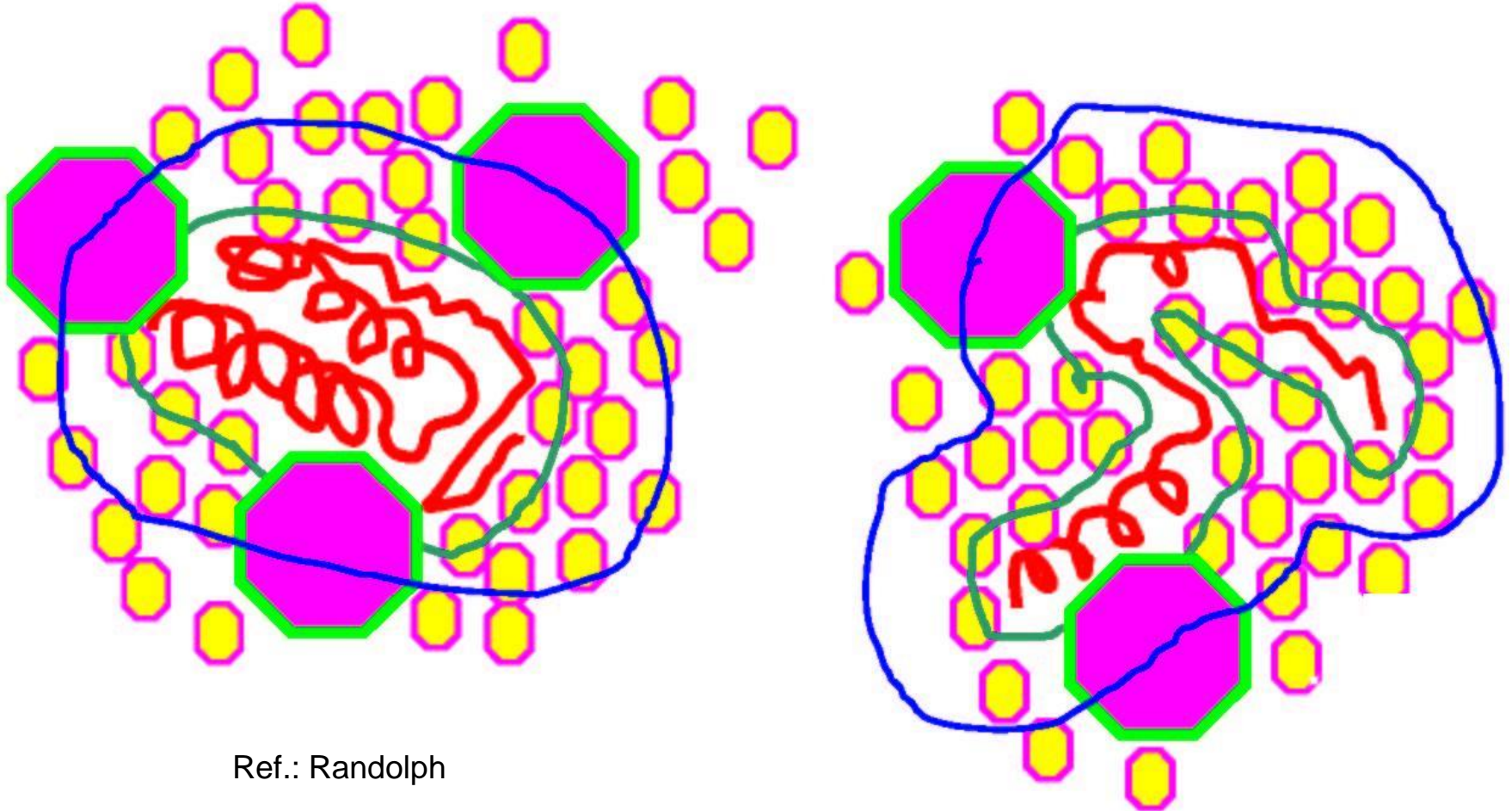
Ref.:Randolph

Native State (N):  
compact, surface area small

Unfolded State (D):  
expanded, surface area high

## Protein(P) - Water(W) – Sugar(S) Interactions

S: Adsorption, Desorption upon unfolding of protein.



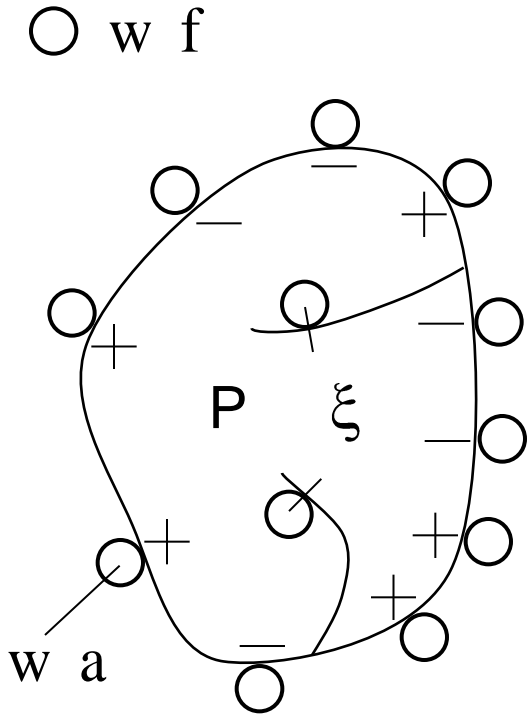
Ref.: Randolph

SW: Coadsorption on surface may stabilize (P).



# Hydratization Process of Proteins (E4)

## Water Intrusion



Stimulus: Chemical potential of water:  $\mu = \mu(p, T, \dots)$

Response: Adsorption of water on P

$$A: n = n(\mu, T = \text{const}) = n_0 + H_0 \left( \mu - \mu_0 \right) + O(2)$$

Number of Adsorption sites:  $\xi$  ... Internal variable!

a)  $\xi = \xi_E = \text{const}$  ... equilibrium :  $\xi = \xi_E$  ( $n, T = \text{const}$ )

b)  $\xi \neq \xi_E$  ... variable ... non-equilibrium:

$$A = A(n, T = \text{const}, \xi) \neq 0$$

Affinity: Measure for non-equ. deviation.

Water:

$$T, p, \mu_w^f = \mu_w^a = \mu$$

# Hydratization Process of Proteins (Water Intrusion)

## Thermostatics 1

Free energy of (P, w)-system:

$$F = F(n, \xi, T) = -SdT + \mu dn - A d\xi, \quad T = \text{const}$$

$$\mu = \left( \frac{\partial F}{\partial n} \right)_{T, \xi} = \mu(n, \xi, T) \quad \dots A1$$

$$-A = \left( \frac{\partial F}{\partial \xi} \right)_{T, n} = -A(n, \xi, T) \quad \dots \text{IEOS}$$

External & internal or full equilibrium:  $F \rightarrow \text{Min}$ ,  $T = \text{const}$ ,  $n = \text{const}$

$$A(n, \xi, T) = 0 \rightarrow \xi_E = \xi_E(n, T) = \text{const}$$

External equilibrium only (restricted equilibrium),  $T = \text{const}$ :

$$A \neq 0 \quad \xi \dots \text{arbitrary value}$$

## Hydratization Process of Proteins (System: P, w(a))

Free Energy, Taylor Series

$$F(n, \xi, T) = F_{00} + F_{10}n + F_{01}\xi + \frac{1}{2!} F_{20}n^2 + 2F_{11}n\xi + F_{02}\xi^2 + O(3)$$

Thermodynamic Stability (2<sup>nd</sup> Law):  $\left\| \partial^2 F / \partial n \partial \xi \right\| > 0$ ,  $F_{ik} = F_{ki}$  T

$$\rightarrow F_{20} \geq 0, \quad F_{20}F_{02} - F_{11}^2 > 0, \quad F_{02} \geq 0$$

Reference State:  $Z_0(n_0, \mu_0, \xi_0, A_0 = 0, T)$

Equations of State:

$$\mu = \left. \frac{\partial F}{\partial n} \right|_{\xi, T} : \quad \mu - \mu_0 = F_{20}(n - n_0) + F_{11}(\xi - \xi_0) \quad 1$$

$$-A = \left. \frac{\partial F}{\partial \xi} \right|_{n, T} : \quad -A = F_{11}(n - n_0) + F_{02}(\xi - \xi_0) \quad 2$$

Internal Equilibrium:  $A(n, \xi_E, T) = 0$ ,  $\xi_E - \xi_0 = -\frac{F_{11}}{F_{02}}(n - n_0)$

$$\underline{\underline{1}} : n - n_0 = H(\mu - \mu_0), \quad H = \frac{F_{02}}{F_{20}F_{02} - F_{11}^2} > H_0 = \frac{1}{F_{20}}$$

# Hydratization Process of Proteins (System: P, w(a))

## Thermodynamics of Processes

$$1^{st} \text{ Law: } dU = dQ + h dn + 0$$

$$2^{nd} \text{ Law: } dS = \frac{1}{T} dU - \frac{\mu}{T} dn + \frac{A}{T} d\xi$$

$$dS = \frac{Q}{T} + s dn + dS_{in}$$

$$\mu = h - Ts$$

$$P_s = \dot{S}_{in} = \frac{A}{T} \dot{\xi} \geq 0$$

$$\text{Eckart-Onsager: } \Delta \dot{\xi} = \alpha n, \xi, T \quad A + O \quad A^2$$

$$\text{Equations of State: } \Delta\mu = F_{20}\Delta n + F_{11}\Delta\xi$$

$$-A = F_{11}\Delta n + F_{02}\Delta\xi$$

} \*

$$\Delta\mu \quad t = \mu - \mu_0 \rightarrow \Delta n \quad t = n - n_0, \quad \Delta\xi \quad t = \xi - \xi_0, \quad A = A \quad t \rightarrow 0!$$

Stimulus

Adsorption

Structure

Equilibrium

## Hydratization Process of Proteins (System: P, w(a))

$$\text{Stimulus} : \Delta\mu = \mu_{p,T,\dots} - \mu_0$$

$$\text{Adsorption: } \Delta n = n_t - n_0$$

$$\text{Structure} : \Delta\xi = \xi_t - \xi_0 \dots \text{ adsorption sites}$$

$$\tau_n \Delta\dot{\mu} + \Delta\mu = E \Delta n + \tau_\mu \Delta\dot{n} \quad (\text{Poynting, Elastic Relax.})$$

$$* \quad \tau_n^{-1} = \alpha F_{02} > 0, \quad E = F_{20} - \frac{F_{11}^2}{F_{02}} \geq 0, \quad \tau_\mu^{-1} = \left( F_{02} - \frac{F_{11}^2}{F_{20}} \right) \alpha > 0$$

$$\tau_n < \tau_\mu$$

### Adsorption Process

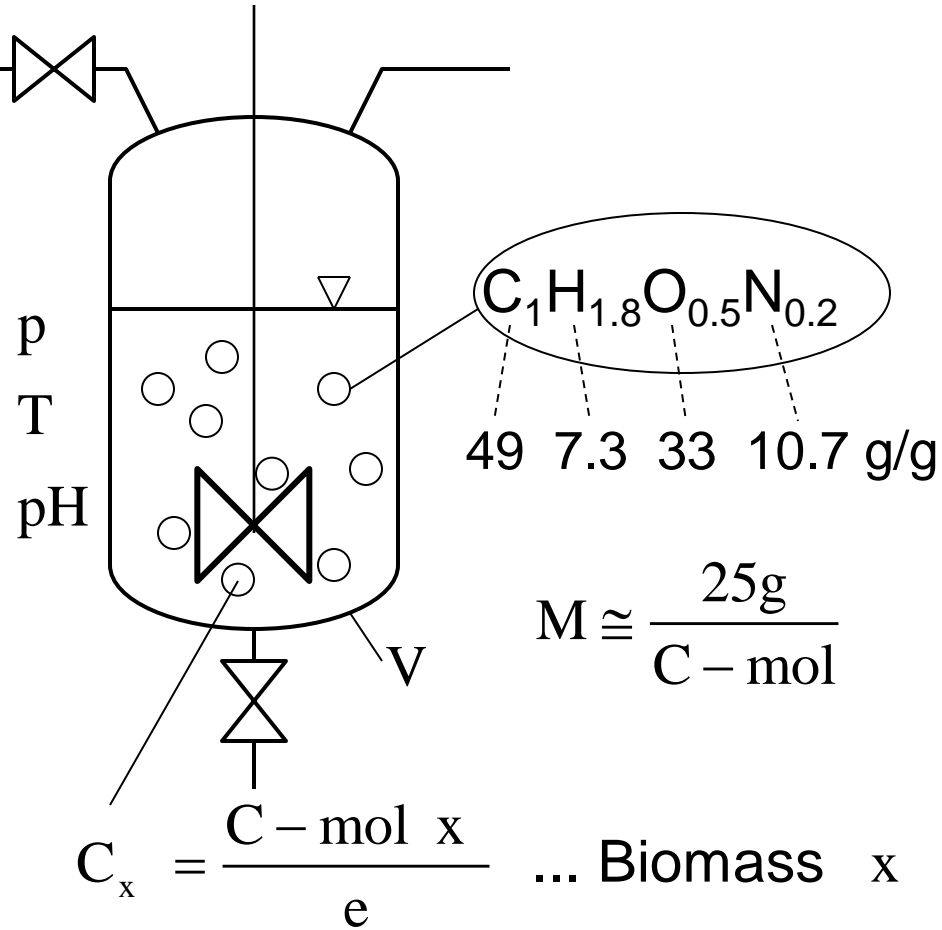
$$\Delta n_t = \frac{1}{\tau_\mu E} \int_0^t ds \left[ \Delta\mu_s + \tau_n \Delta\dot{\mu}_s \right] e^{-t-s/\tau_\mu} ds$$

### Protein structure / Adsorption sites

$$\Delta\xi_t = \frac{1}{F_{11}} \left\{ \Delta\mu - \alpha F_{20} \int_0^t ds \left[ \Delta\mu_s + \tau_n \Delta\dot{\mu}_s \right] \right\} e^{-t-s/\tau_\mu} ds$$

# 5A. Metabolism of Living Bacteria\*

Fermenter



## Example (Yeast)

Genes	5000
Metabolites	1000-5000
Concentration <sup>*)</sup>	0.1–10 mmol
Turn over time	
$\frac{\text{Concentration}}{\text{Reaction rate}}$	= 1–10 s

<sup>\*)</sup> Osmotic pressure limited.  
Avoiding byproducts and byreactions.

\*Microbiothermodynamic system, Microbioreactor

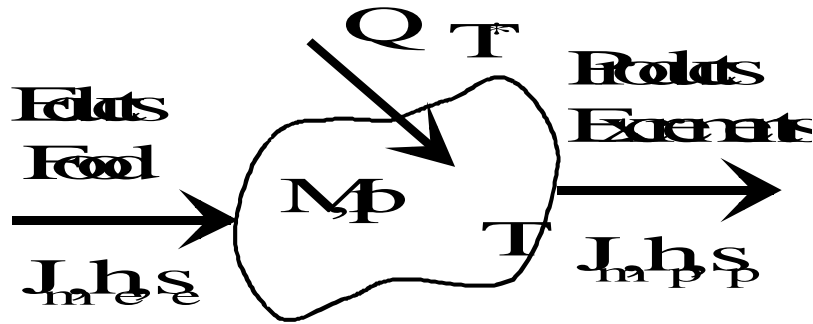


**Bacteria Stylonychia (Wimpertierchen / Eyelash bacteria)**

# Mesoscopic Biofluids / Bacterial Solutions

## Exergy Analysis of Microbioreactors (MBRs)

### Stationary States



$$1^{st} \text{ Law: } \dot{U} = (h_e - h_p)J_m + \dot{Q} = 0 \quad (1)$$

$$2^{nd} \text{ Law: } \dot{S} = (s_e - s_p)J_m + \frac{\dot{Q}}{T^*} + P_s = 0 \quad (2)$$

$$\text{Exergy: } \dot{E} = (e_e - e_p)J_m + \left(1 - \frac{T^*}{T}\right) \dot{Q} + P_{ex} = 0 \quad (3)$$

$$e_i = h_i - h_i^* - T^*(s_i - s_i^*), \quad i = e, p \quad (4)$$

$$(1-3) \quad \frac{e_p - e_e}{h_p - h_e} \leq 1 - \frac{T^*}{T} \quad (5) \quad \begin{matrix} \text{irr. cell} \\ \text{rev. cell} \end{matrix} \quad \rightarrow \quad e_p \leq e_e + \left(1 - \frac{T^*}{T}\right) h_p - h_e \quad (6)$$

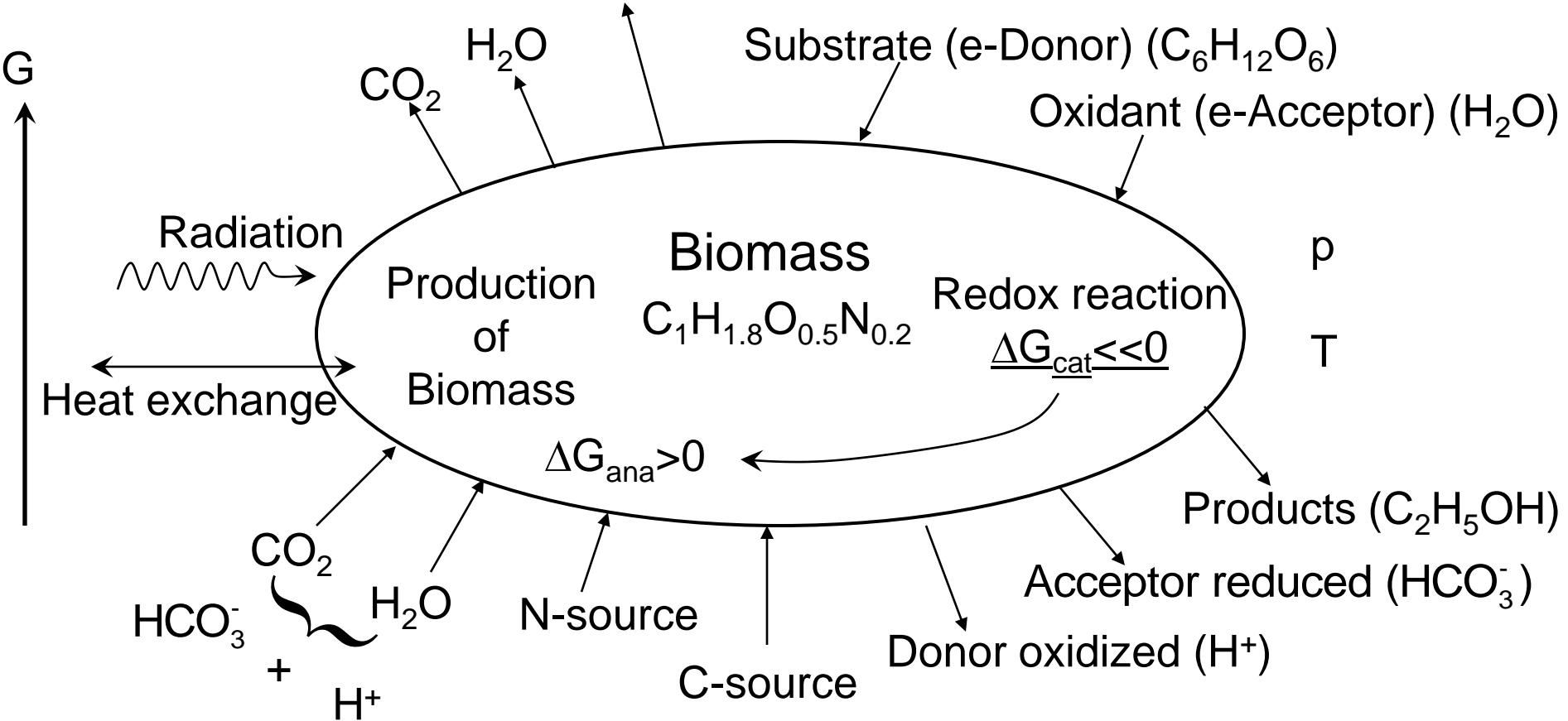
$$\text{COP of MBRs: } \eta_{BR} = \frac{e_p}{e_{pmax}} \stackrel{(6)}{=} \frac{e_p}{e_e + \left(1 - \frac{T^*}{T}\right) h_p - h_e} \leq 1 \quad (7)$$

(5-7): All bacteria, all metabolisms, any temperature and pressure!



# Microbial Growth System

Biomass ( $X=C_1H_{1.8}O_{0.5}N_{0.2}$ )



Anabolism

+

Catabolism (Free Entalpy)

# 5B Thermodynamic Limits of Life

Allometry  
Metabolic Rate

$$\Gamma = a T, T_0 M^\gamma$$

$$a \cong (1 - 2)mW / g$$

$$\frac{2}{3} < \gamma \leq 1$$

$$\gamma \cong \frac{3}{4}$$

B. Ahlborn, Zoological Physics

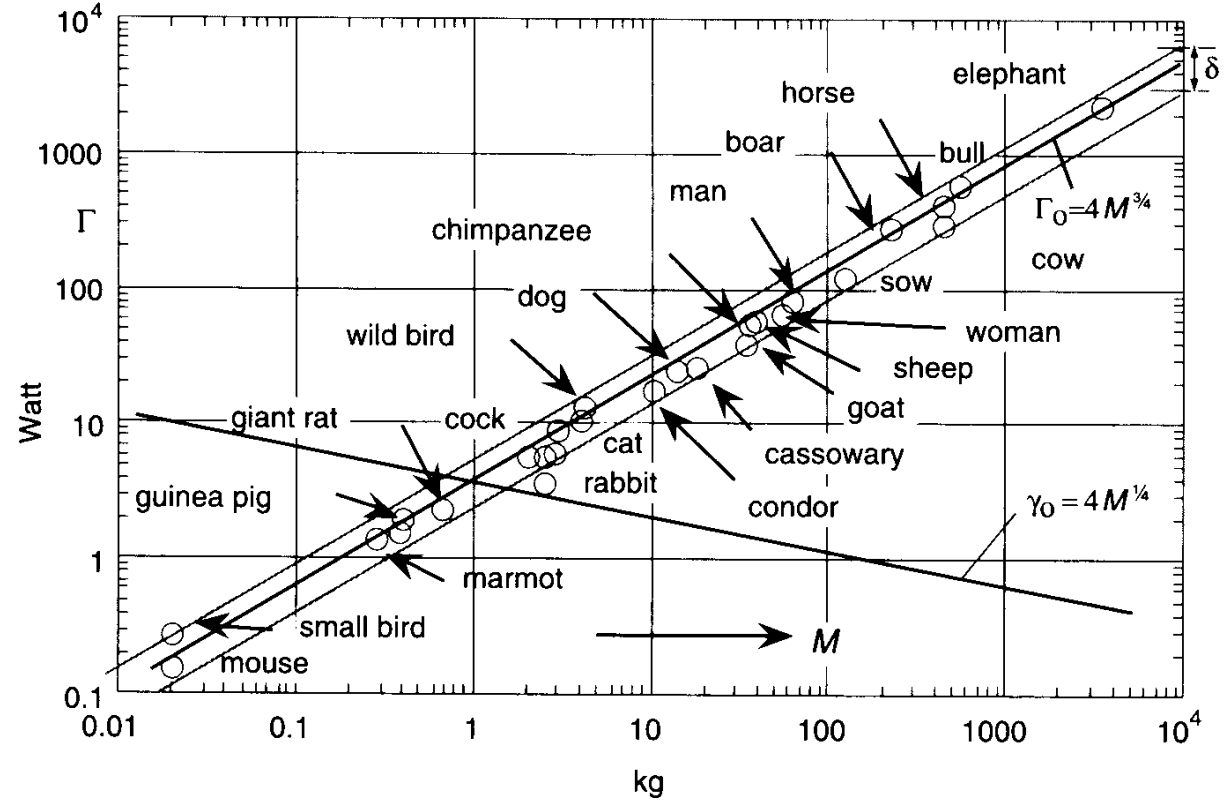


Figure A3  
Metabolic rate of oxygen consumption based living systems. Mouse-Elephant-curve, B. Ahlborn, 2004. This curve also holds for bacteria ( $M \cong 10^{-4}$  g).

# Allometric Constant (a), Temperature Dependence

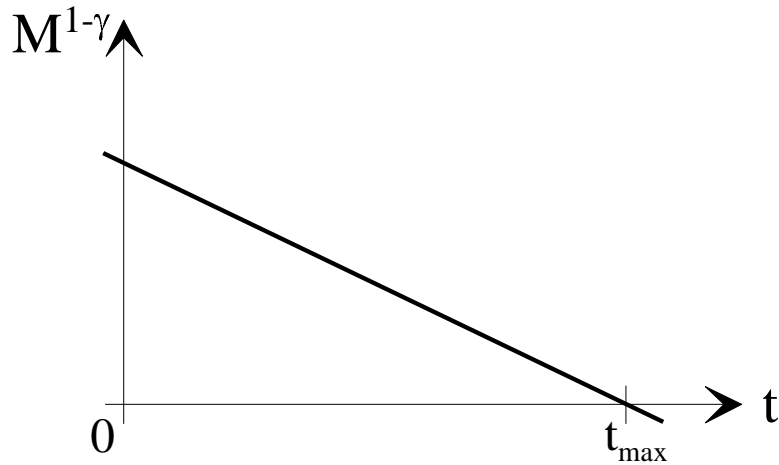


Figure A5  
Mass reduction during autometabolism process of an organism.

$$a(T, T^*) = a_0(T - T^*)e^{-q^*/RT^*}$$

$$a(T = T^*) = 0$$

$$a(T, T^* = 0) = 0 \dots \text{all } (T)$$

1<sup>st</sup> Law      $\dot{Q} = (h_e - h_p)J_m$

Allometry      $\dot{Q} = a(T, T^*)M^\gamma$

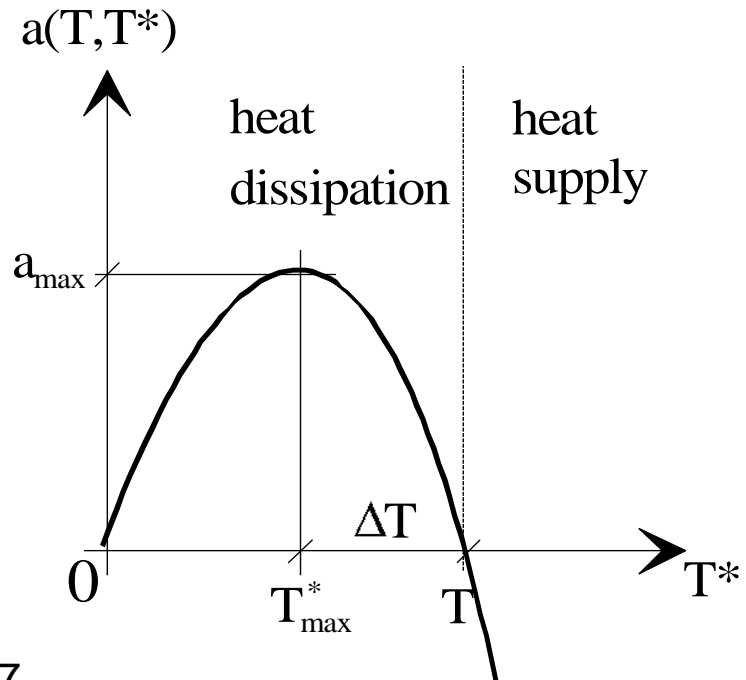


Figure A7  
Dependence of the allometric constant (a) on the environmental temperature ( $T^*$ ) of the bacteria.

# Environmental Temperature for Maximum Metabolism at Given (M, T). System: Fig. A5

$$\Gamma = a(T, T^*) M^\gamma$$

$$a(T, T^*) \rightarrow \text{Max.}$$

$$\rightarrow T^* = T - \frac{q}{R} = T - \Delta T$$

$$\dot{Q}_{\text{max}} = a_0 \Delta T e^{-\frac{\Delta T}{T^*}} M^\gamma$$

$$J_{\text{max}} = \dot{Q}_{\text{max}} / (h_e - h_p)$$

$$\frac{J_{\text{max}}}{M} = \frac{a_0 \Delta T}{h_e - h_p} M^{-1+\gamma} \cdot e^{-\frac{\Delta T}{T^*}}$$

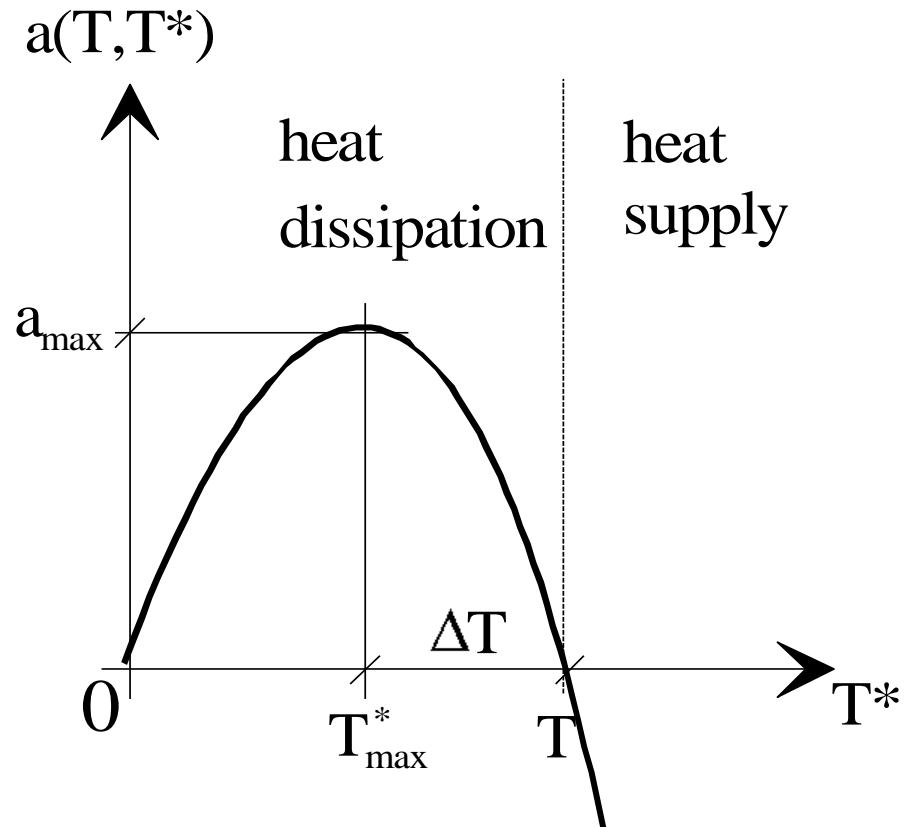
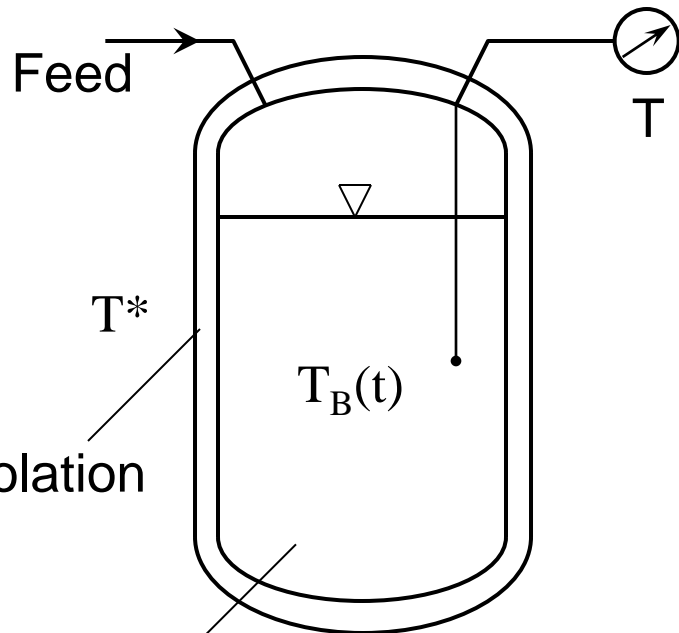


Figure A7  
 Dependence of the allometric constant (a) on the environmental temperature ( $T^*$ ) of the bacteria.

## 6. Biocalorimetry

### Bacterial Identification by Caloric Measurements of Growth Processes (E5)

#### Adiabatic Calorimeter



Metabolic heat

$$dQ = C_B \, n, \dots \, dT_B \quad (1)$$

$$Q(t) = C_B [T_B(t) - T_0] \quad (1A)$$

Metabolic generation of heat:

$$dQ \cong -dn_s \quad (2)$$

$$Q(t) = K [n_{s_0} - n_s(t)] \quad (2A)$$

---


$$n_s(t) = n_{s_0} - \frac{Q(t)}{K} \quad (2B)$$

Broth: Water ( $n_W$ )  
 Substrate ( $n_S$ )  
 Bacteria ( $n$ )  
 .....

# Bacterial Identification by Caloric Measurements

Bacterial growth process:

$$dn \cong n_s n dt \quad (3)$$

$$\frac{dn}{n} = A n_s(t) dt \quad (3A)$$

$$(2B) \quad \frac{dn}{n} = A \left[ n_{s_0} - \frac{Q(t)}{K} \right] dt$$

$$(1A) \quad n(t) = n_0 \exp \left\{ A \int_0^t \left[ n_{s_0} - \frac{C_B}{K} T_B(s) - T_{B_0} \right] ds \right\} \quad (3B)$$

Bacterial population

Measurement

Process model: Monod

$$n(t) = n_0 + n_\infty - n_0 \frac{b t^\alpha}{1 + b t^\alpha} \quad (4)$$

$$(3B, 4) \rightarrow \alpha, b = \frac{1}{t_B}$$

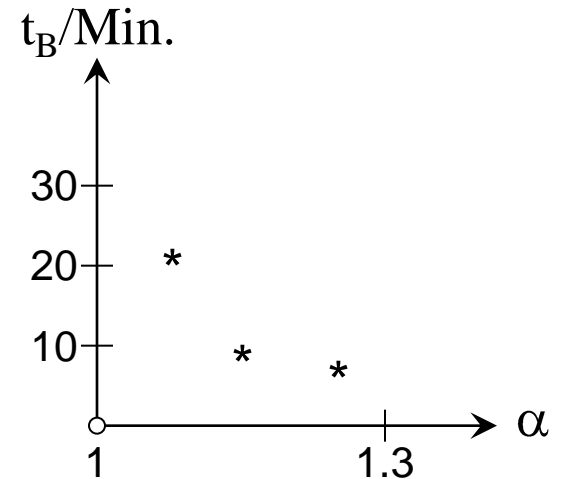
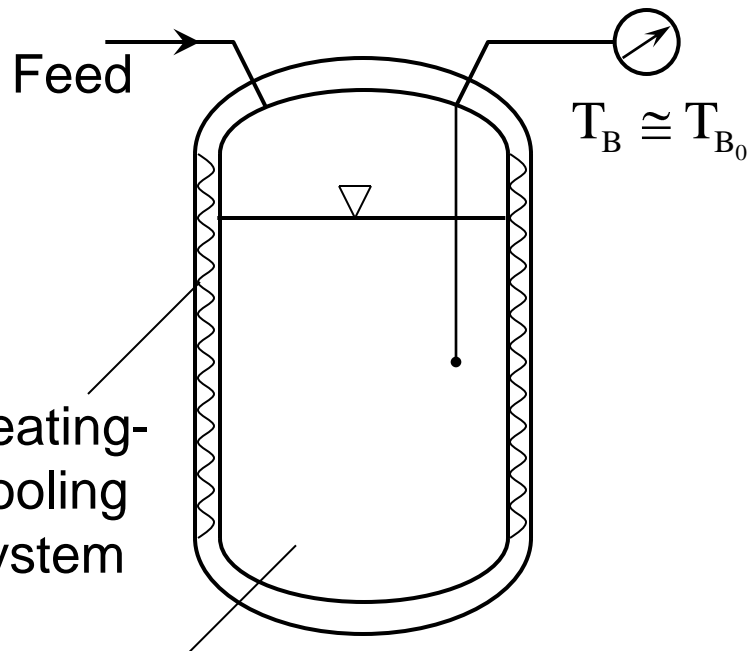


Diagram of characteristic parameters ( $\alpha, t_B=1/b$ )

# Bacterial Identification by Caloric Measurements of Growth Processes

## Isothermal Calorimeter



Broth: Water ( $n_W$ )  
 Substrate ( $n_S$ )  
 Bacteria ( $n$ )  
 .....

Metabolic generation of heat:

$$dQ \cong -dn_S \quad (2)$$

Compensational heat (Peltier)

$$dQ_C = \Pi I^2 dt \quad (5)$$

Isothermal condition

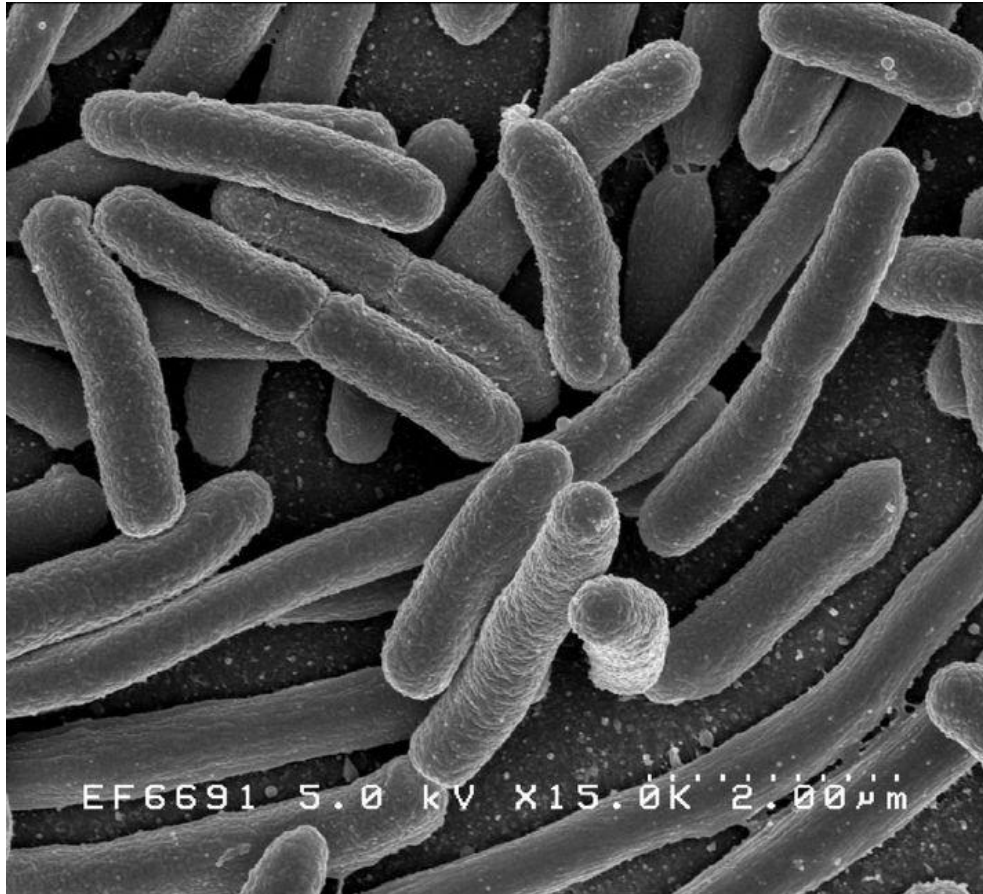
$$T_{B_0}: \quad 0 = dQ + dQ_C \quad (6)$$

Bacterial growth measurement (3)

$$n(t) = n_0 \exp \left\{ A \int_0^t \left( n_{S_0} + \frac{Q_C(s)}{K} \right) ds \right\}$$

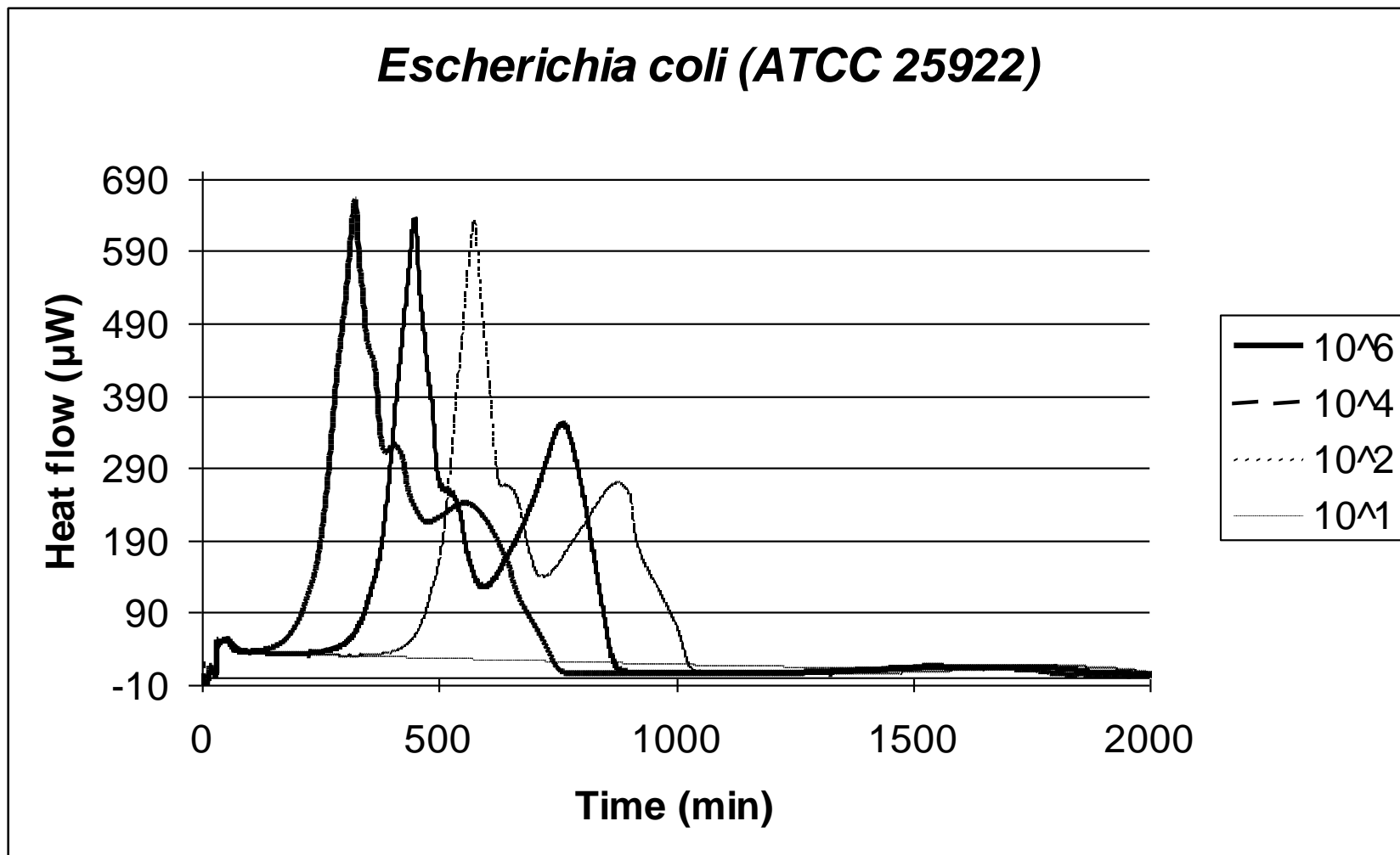
Model (monod)

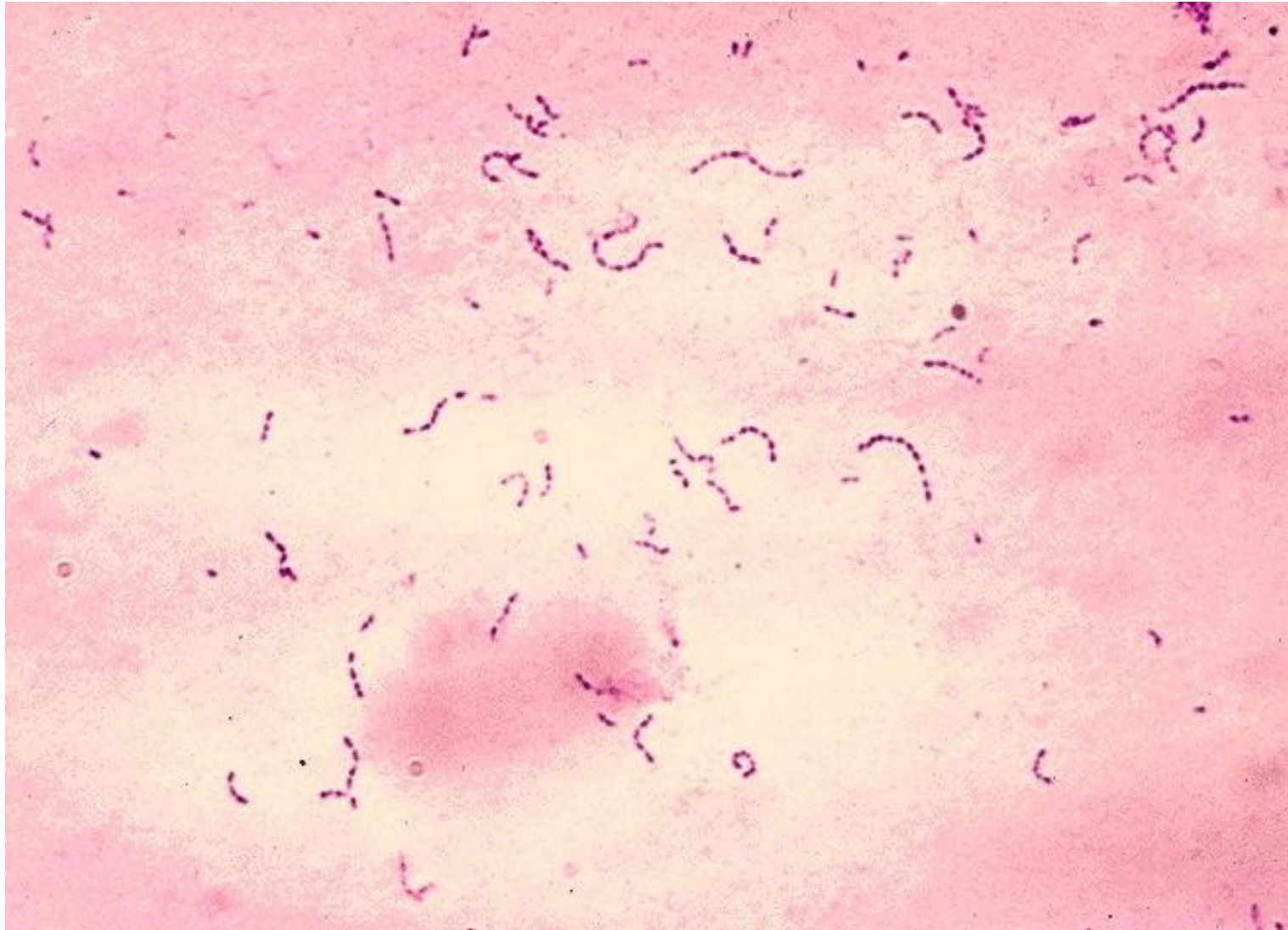
$$(4) \quad n(t) = n_0 + (n_\infty - n_0) \frac{(bt)^\alpha}{1 + (bt)^\alpha} \rightarrow (\alpha, b)$$



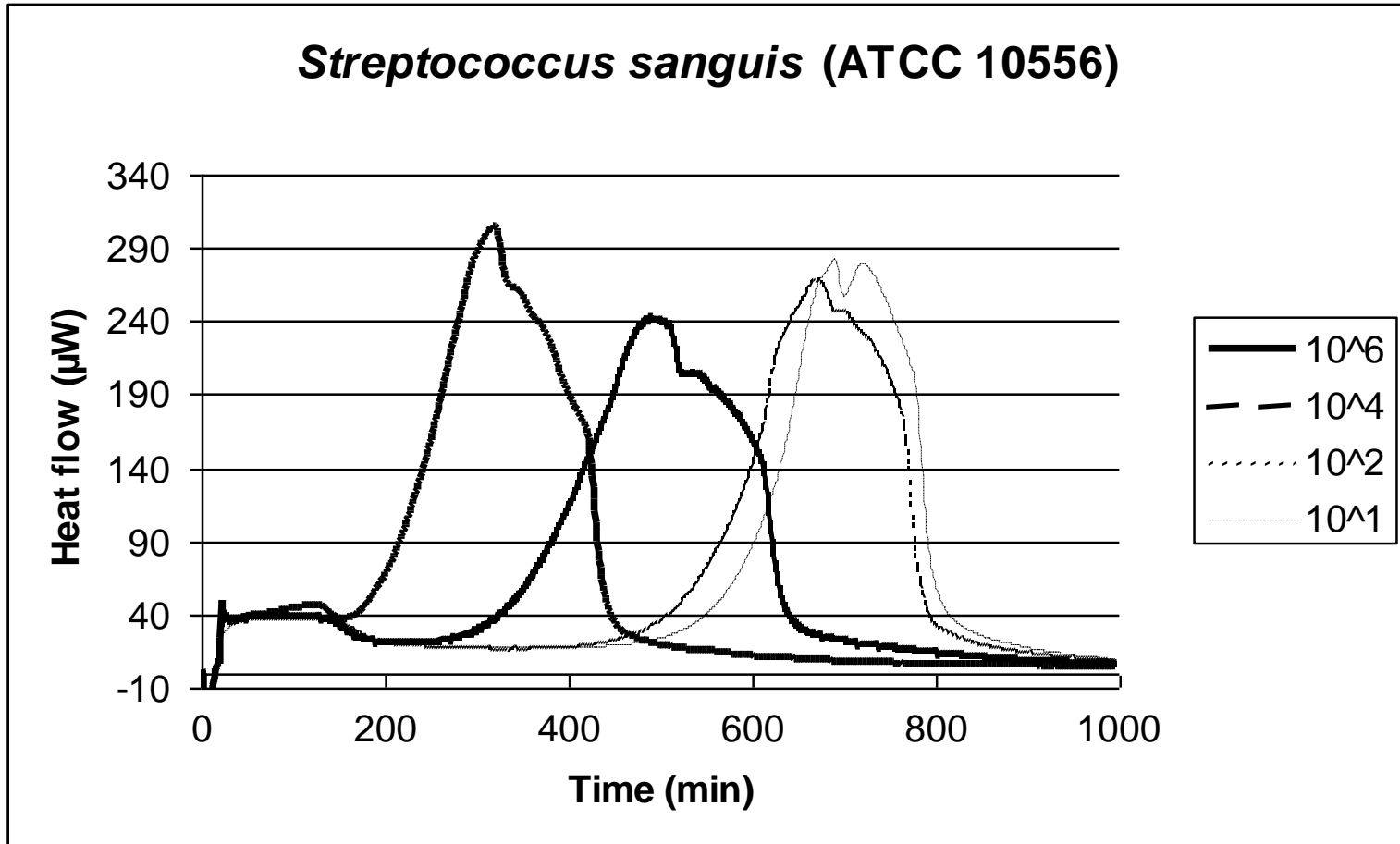
**Bacteria Escherichia coli, Th. Escherich (1919)**







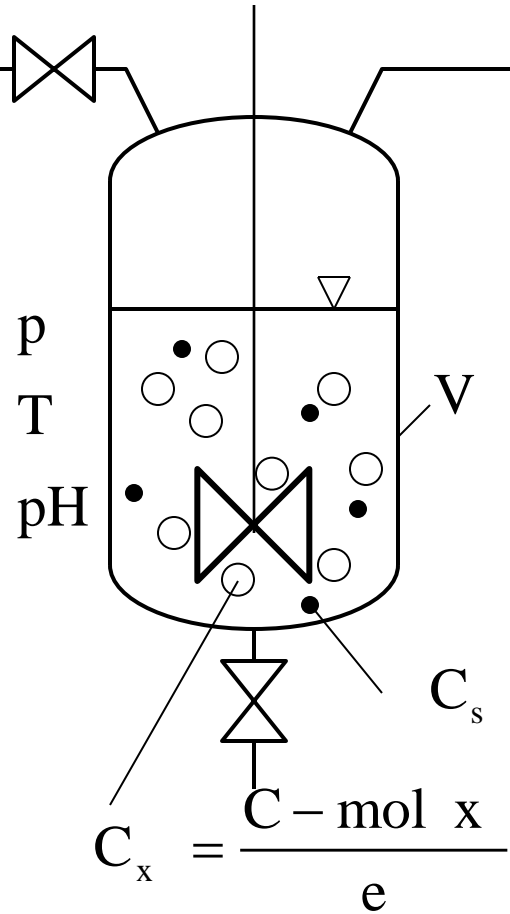
**Bacteria Streptococcus Mutans (Karies), Clarke (1924)**



# 6. Bioreactors

## Microbial Growth at Constant Substrate Concentration

Fermenter



Rate equation

$$dC_x = \mu_x C_x dt$$

Growth rate<sup>\*)</sup>

$$\mu_x = \frac{C - \text{mol } x}{C - \text{mol } x \cdot h}$$

0.001–2

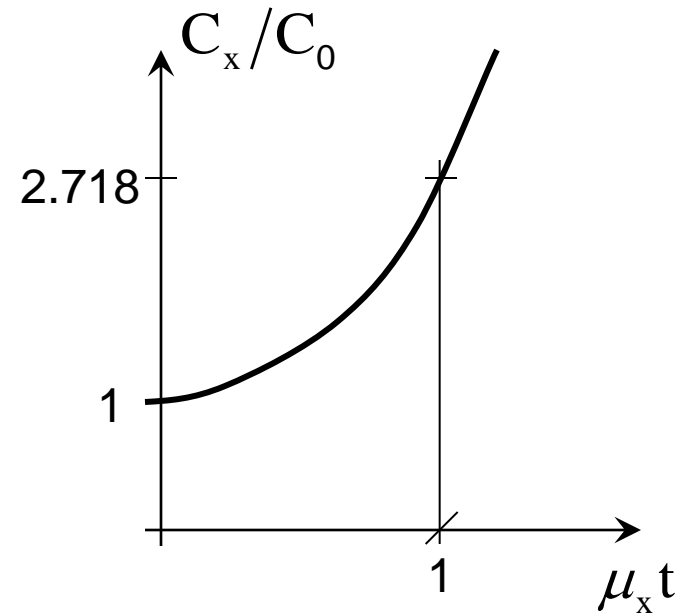
$$C_s = \frac{\text{mol } s}{e} \dots \text{Substrate } s$$

$$C_x = \frac{C - \text{mol } x}{e} \dots \text{Biomass } x$$

Microbial growth

$$C_x t = C_0 e^{\mu_x t}$$

$$\mu_x = \text{const} = \frac{1}{\tau}$$

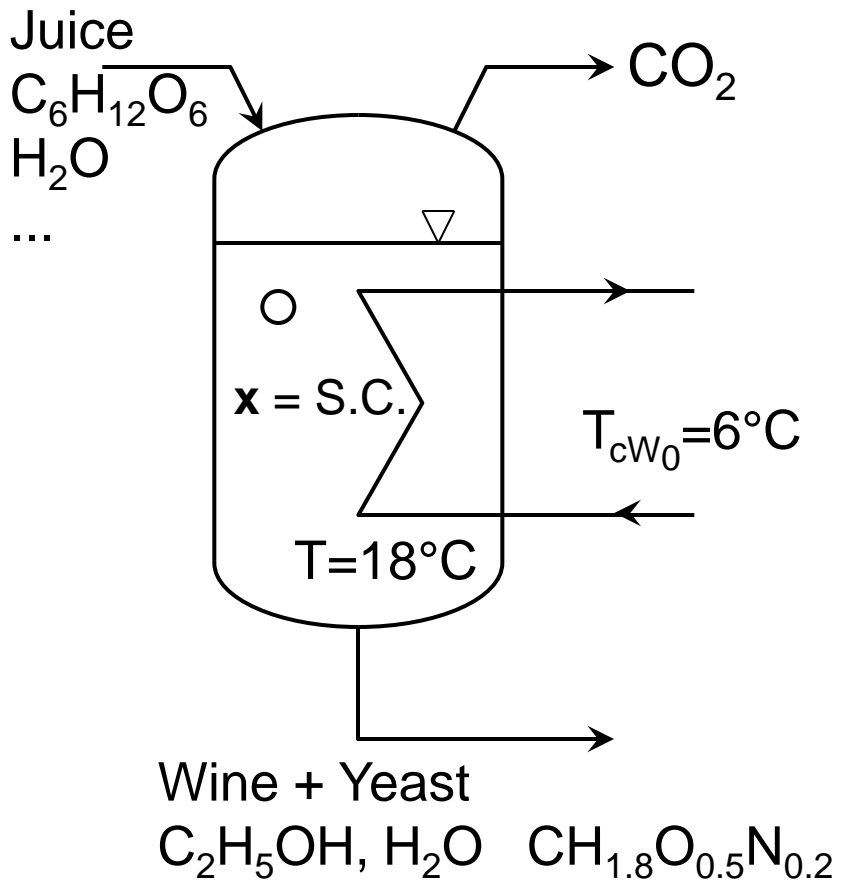


<sup>\*)</sup>Limited by  $\bar{e}$ -transport

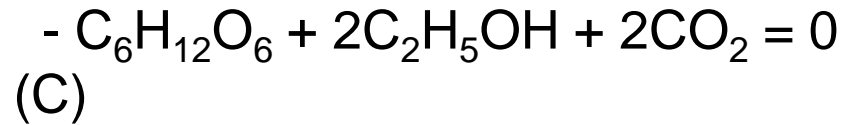
capacity in cell membranes:  $3 \text{ mol } \bar{e} / C - \text{mol } x \text{ h } 298 \text{ K}$

# Wine Fermentation, Heat Production, Cooling Process (1)

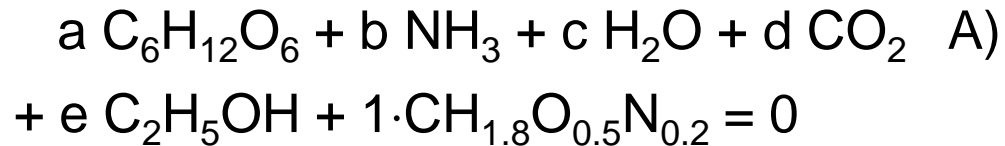
## Problems: Oxygen, Pressure, pH-Value



Catabolic reaction



Anabolic fermentation reaction



Anabolic growth (Experiment)

$$Y_{GLUX} = 0.057 \frac{C - mol \ x}{C - mol \ GLU}$$

**x = S.C.:** Saccharomycae cervisiae  
 Wine yeast (Weinhefe)

## Wine Fermentation, Heat Production, Cooling Process (2)

### Biological parameters

$$Y_{\text{GLUX}} = 0.057 \frac{\text{C-mol } x}{\text{C-mol GLU}}$$

### Growth rates

$$\mu_x = 0.05 \frac{\text{g}}{\text{g h}} \dots 18^\circ\text{C}$$

$$\mu_x = 0.34 \frac{\text{g}}{\text{g h}} \dots 30^\circ\text{C}$$

### Molar mass (including ash)

$$M_x = 26 \frac{\text{g}}{\text{C-mol } x}$$

### Enthalpy of combustion

$$\Delta h_x = -472 \frac{\text{kJ}}{\text{C-mol } x}$$

### Initial yeast concentration

$$n_x = 0.2 \text{ C-mol } x / \text{l}$$

### Oenological parameters

#### Juice

$$c_{\text{GLU}_0} = 210 \frac{\text{g}}{\text{l}}$$

$$M_{\text{GLU}} = 180 \frac{\text{g}}{\text{mol}}$$

$$v_{\text{GLU}_0} = 1.167 \frac{\text{mol}}{\text{l}}$$

$$v_{\text{GLU}_\infty} = 0$$

$$\rho_J = 1.0 \text{ kg/l} = \rho_{\text{Wine}}$$

$$c_{pJ} = 4.186 \frac{\text{kJ}}{\text{kg K}} = c_{p\text{Wine}}$$

### Technical parameters

$$V = 10000 \text{ l}$$

$$T = 18^\circ\text{C}$$

$$T_{cW_0} = 6^\circ\text{C}$$

### Heat transfer

$$k = 200 \frac{\text{W}}{\text{m}^2\text{K}}$$

## Wine Fermentation

### Problems

1. Stoichiometry of anabolism  
Heat production
2. Stoichiometry of catabolism  
sugar  $\rightarrow$  alcohol
3. Pressure dependence
4. Heat balance of reactor
5. Maximum heat production rate
6. Heat exchange area  
Tube length, cooling water flow

## Thermodynamic Data

### Heat of combustion

(25°C, 1atm, pH=7)

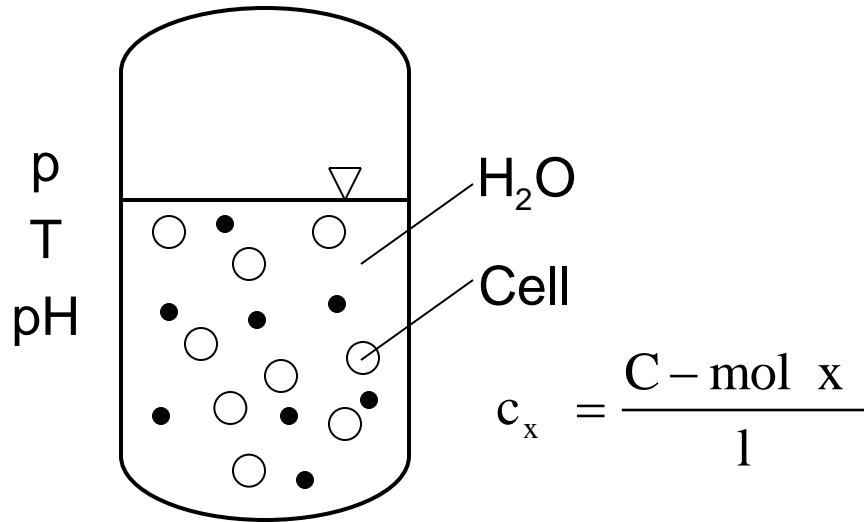
Glucose ( $C_6H_{12}O_6$ ) -2813.6 kJ/mol

Ethanol ( $C_2H_5OH$ ) -1356.8 kJ/mol

Biomass ( $CH_{1.8}O_{0.5}N_{0.2}$ ) -475kJ/mol

$CO_2, NH_3, H_2O$   $\emptyset$  kJ/mol

# Phenomenological Kinetics of Cell Death (Sterilization Processes)



$$1 \quad c_x \quad t = c_{x_0} e^{-k_d t}$$

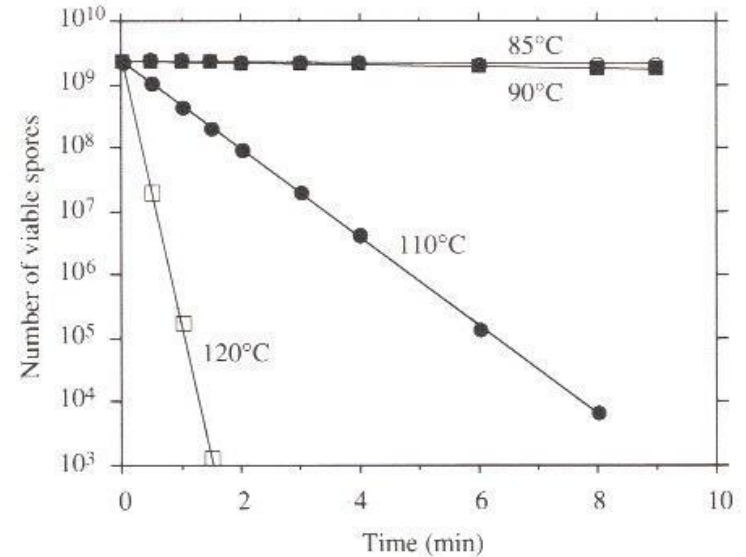
$$\underline{\ln c_x = \ln c_{x_0} - k_d t} \quad 2$$

Cell death  $\approx$  Enzyme deactivation  
loss of viability

$$\dot{c}_x = -k_d c_x \quad 1$$

$$k_d \quad T = k_{d_0} e^{-\frac{E_d}{R} \left( \frac{1}{T} - \frac{1}{T_0} \right)}$$

$$E_d = 250 - 300 \text{ kJ/mol}$$

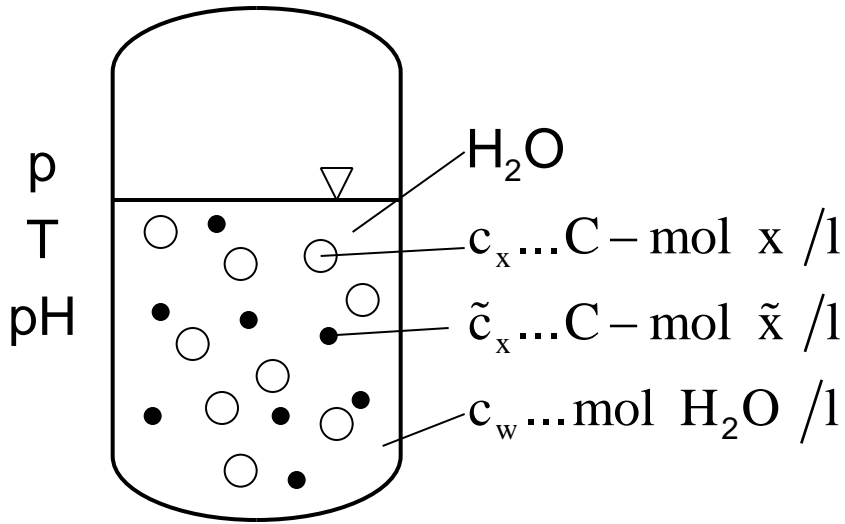


Thermal death of *Bacillus subtilis* spores.

T/°C	85	90	110	120
$k_d/\text{min}^{-1}$	0.012	0.032	1.60	9.61



# Thermodynamics of Cell Death Processes\*) (T-Dependence)



Death reaction:  $x \rightarrow \tilde{x}$

$$G = G(n_x, \tilde{n}_x, n_w, T, p)$$

$$p, T : dG = \mu_x dn_x + \tilde{\mu}_x d\tilde{n}_x \quad 1$$

Mass balance (C-Atoms)

$$d\tilde{n}_x = -dn_x \Big|_{\frac{1}{V}}$$

$$\tilde{c}_x = \tilde{c}_{x_0} - c_x - c_{x_0} \quad 2$$

\*) Analogy: Radioactive gas.

$$1,2 \quad dg = \overbrace{\mu_x - \tilde{\mu}_x}^{\text{Force Flux}} dc_x < 0 \quad \dots 2^{\text{nd}} \text{ Law}$$

$$\dot{c}_x = F \frac{\mu_x - \tilde{\mu}_x}{\mu_x - \tilde{\mu}_x} \quad 3$$

1<sup>st</sup> order kinetics

$$\dot{c}_x = -k_d c_x \quad 4$$

Thermostatics of ideal solutions

$$\tilde{\mu}_x = \tilde{\mu}_{x_0}(T, p) + RT \ln \tilde{x}_x, \quad \tilde{x}_x = \frac{\tilde{c}_x}{c_x + \tilde{c}_x + c_w}$$

$$\mu_x - \tilde{\mu}_x = \mu_{x_0} - \tilde{\mu}_{x_0} + RT \ln \left( \frac{c_x}{\tilde{c}_x} \right) \quad 5$$

$$2-5 \quad \dot{c}_x = -k_d \frac{c_{x_0} \exp\left(\frac{\mu_x - \tilde{\mu}_x}{RT}\right)}{K + \exp\left(\frac{\mu_x - \tilde{\mu}_x}{RT}\right)} = -k_d c_x \quad 6$$

$$K(T) \exp\left(\frac{\mu_{x_0} - \tilde{\mu}_{x_0}}{RT}\right) = F \mu_x - \tilde{\mu}_x$$

## Production of Biomacromolecules\*

### Upstream Processing 25 C, \$: 20%-30%

### Downstream Processing -200C – 150C, \$:70%-80%

Genetic engineering  
Genomics, Proteomics

Selection of  
Protein encoding  
Gene

Cell disruption

Centrifugation

Microbiology  
Bacteria, Fungi, Cells

Selection of  
Microbioreactor

Ultracentrifugation

Fermentation

Cell production

**Chromatography**

High resolution

Purification

Cell harvesting

Product / Formulation (pH)

\*Recombinant proteins, DNAs, Ref.Tosoh Bioscience GmbH, Voet&Voet, Biochemistry

# Problems in Downstream Processing of Biological Fluids

Parameters	Non-Biological Fluids	Downstream Processing Fluid
Number of Compounds	Low	Very high (>1000)
Pure State Data	Available	Difficult
Interactions	similarities	Whole spectrum (Coulomb, v.d.Waals)
Molecular Weight	comparable	Very different, from low to very high
Model Description	Possible with semi-empirical models	No model
Prediction of a Unit Separation	Possible	Presently not Possible

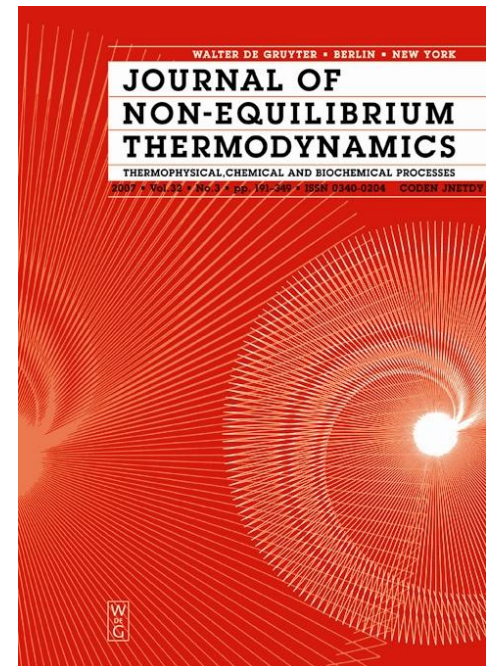
Ref.: Müller E., Tosoh Bioscience GmbH, 2006.

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## **KISS**

Keep it smart and simple.

## **MORENE**

More research needed.

**Ötztaler Alpen, 5-9-2007**

**Similaunhütte, 3012m, (T= -10C / -30C)**

